AN ASSESSMENT OF ANGIOGENESIS AND ELASTOSIS IN FIBROCYSTIC BREAST DISEASE AND INVASIVE BREAST CARCINOMA

Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2015

DECLARATION

I hereby declare that the dissertation entitled **"AN ASSESSMENT OF ANGIOGENESIS AND ELASTOSIS IN FIBROCYSTIC BREAST DISEASE AND INVASIVE BREAST CARCINOMA"** is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from April 2013 to July 2014 under the guidance and supervision of **Dr.A.DHANALAKSHMI M.D.,** Associate Professor, Department of Pathology, Coimbatore Medical College.

This dissertation is submitted to The Tamilnadu Dr.MGR Medical University, Chennai towards the partial fulfilment of the requirement for the award of M.D., Degree (Branch III) in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

Place : Coimbatore

Date : Dr. DURAISAMY.R

CERTIFICATE

This is to certify that dissertation entitled **"AN ASSESSMENT OF ANGIOGENESIS AND ELASTOSIS IN FIBROCYSTIC BREAST DISEASE AND INVASIVE BREAST CARCINOMA"** is a bonafide work done by **Dr.R.DURAISAMY** ,a postgraduate student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore under the supervision and guidance of **Dr.A. DHANALAKSHMI M.D.,** Associate professor, Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore in partial fulfillment of the regulations of the Tamilnadu Dr. M.G.R. Medical University, Chennai towards the award of M.D. Degree (Branch III) in Pathology.

Dr. S.REVWATHY, M.D.,D.G.O.,DNB
Dean,
Coimbatore medical College,
Coimbatore.

Dr. C. LALITHA, M.D.,
Professor and Head of Department,
Department of Pathology,
Coimbatore Medical College,
Coimbatore.

Guide
Dr.A. DHANALAKSHMI. M.D.,
Associate professor,
Department of pathology,
Coimbatore Medical College,
Coimbatore.

Coimbatore Medical College

COIMBATORE, TAMILNADU, INDIA - 641 014

(Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai)

ETHICS COMMITTEE

CERTIFICATE

Name of the Candidate : R. DURAISAMY

Course : M.D. PATHOLOGY

Period of Study : 2012 - 2015

College : COIMBATORE MEDICAL COLLEGE

Dissertation Topic : AN ASSESSMENT OF ANGIOGENESIS AND ELASTOSIS IN FIBROCYSTIC BREAST DISEASE AND INVASIVE BREAST CARCINOMA.

The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation Proposal is accepted / ~~Not accepted~~ and you are permitted / ~~Not permitted~~ to proceed with the above Study.

DEAN
Coimbatore Medical College & Hospital,
Coimbatore

ACKNOWLEDGEMENT

To begin with, I thank the almighty God for bestowing his blessings on me in making this project a successful one.

I wish to express my sincere gratitude to the Honourable Dean **Dr.S.Revwathy M.D.,D.G.O.,DNB.,** Coimbatore Medical College, Coimbatore for Permitting me to conduct this study.

It is a great pleasure to express my humble gratitude to **Dr. C. Lalitha M.D.,** Professor and Head, Department of Pathology, Coimbatore Medical College, Coimbatore for her able guidance and support.

I wish to express my gratitude and sincere thanks to **Dr. Arjunan. A. M.D.,** for his guidance and support.

I express my deepest sense of gratitude and sincere thanks to my guide **Dr.A. DHANALAKSHMI M.D.,** associate professor in Department of Pathology, Coimbatore Medical college, Coimbatore. This dissertation bears imprint of her valuable suggestions and highly professional advice.

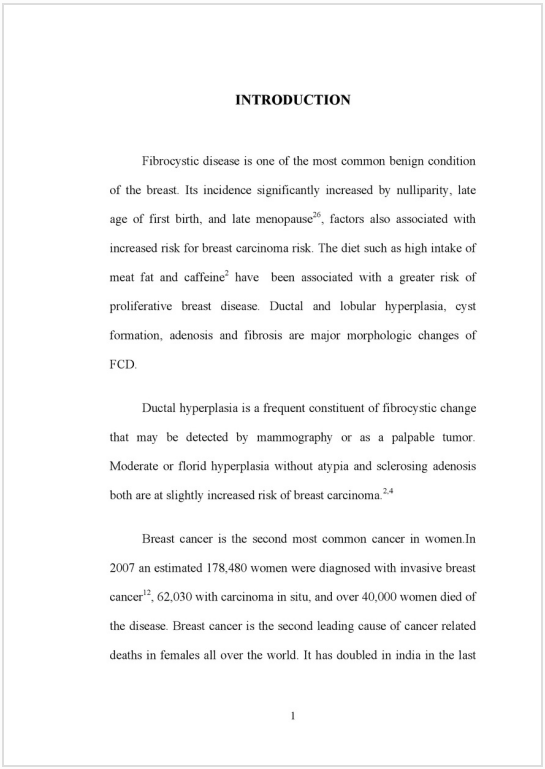
I owe my gratitude to Dr. Triveni Balajji, M.D., and Dr. R. D. Puvitha, M.D., for their timely advice and suggestions through the course of my work.

I thank all Assistant Professors and tutors of Pathology Department, Coimbatore Medical college, Coimbatore for their encouragement and valuable opinion.

I thank, Department of surgery, Coimbatore Medical College, Coimbatore for providing clinical cases, valuable supervision, support and guidance which made my study possible and also thanks to all lab technician working in pathology department.

Finally, I would like to thanks my wife Dr.Sathiya and my brother in law Mr.K.Senthilkumar B.E.

Dr. DURAISAMY.R



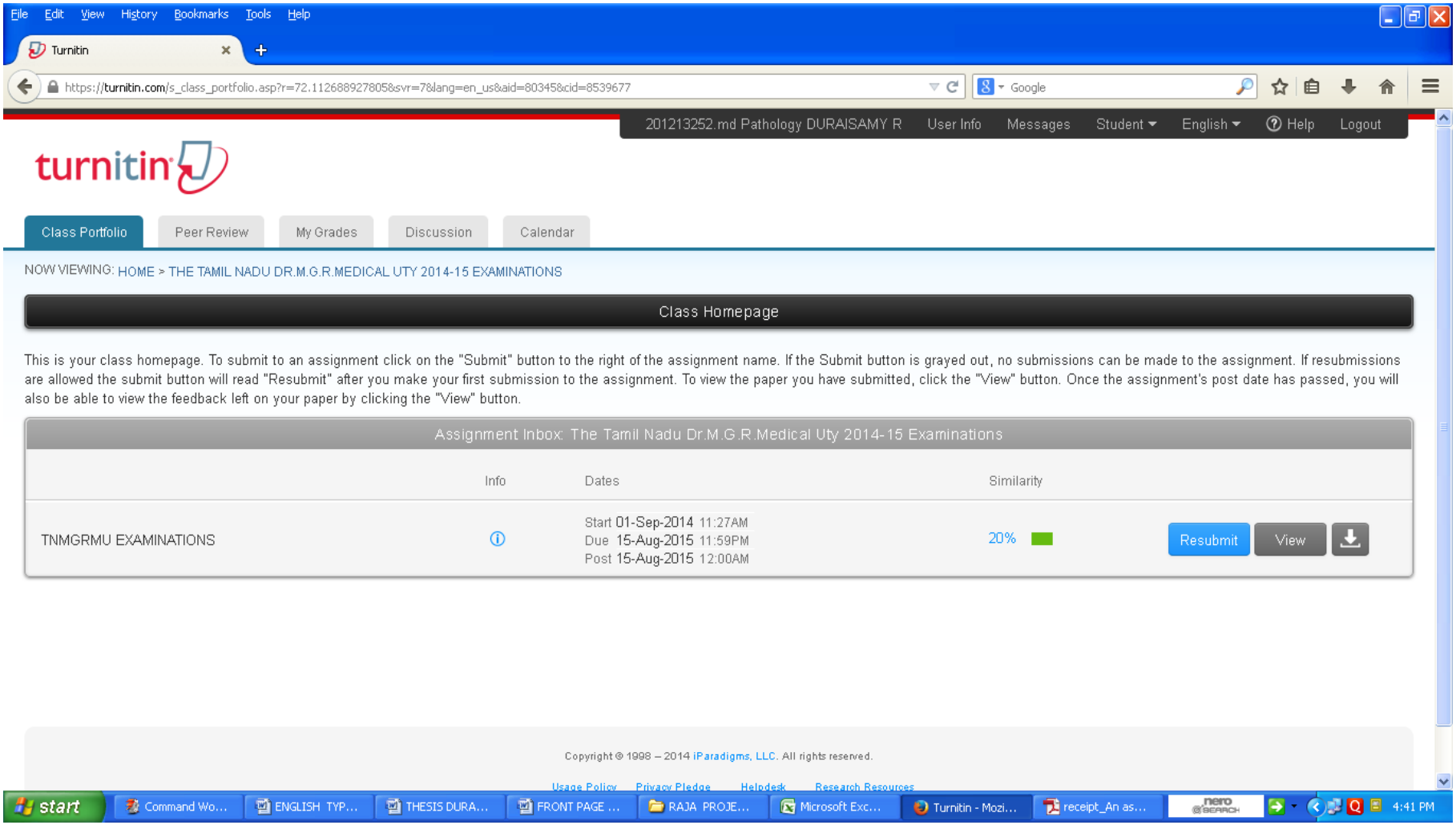
INTRODUCTION

Fibrocystic disease is one of the most common benign condition of the breast. Its incidence significantly increased by nulliparity, late age of first birth, and late menopause[26], factors also associated with increased risk for breast carcinoma risk. The diet such as high intake of meat fat and caffeine[2] have been associated with a greater risk of proliferative breast disease. Ductal and lobular hyperplasia, cyst formation, adenosis and fibrosis are major morphologic changes of FCD.

Ductal hyperplasia is a frequent constituent of fibrocystic change that may be detected by mammography or as a palpable tumor. Moderate or florid hyperplasia without atypia and sclerosing adenosis both are at slightly increased risk of breast carcinoma.[2-4]

Breast cancer is the second most common cancer in women.In 2007 an estimated 178,480 women were diagnosed with invasive breast cancer[12], 62,030 with carcinoma in situ, and over 40,000 women died of the disease. Breast cancer is the second leading cause of cancer related deaths in females all over the world. It has doubled in india in the last

1


File Edit View History Bookmarks Tools Help
Turnitin
https://turnitin.com/s_class_portfolio.asp?r=72.112688927805&svr=7&lang=en_us&aid=80345&cid=8539677
Google
201213252.md Pathology DURAISAMY R
User Info
Messages
Student
English
Help
Logout
turnitin
Class Portfolio
Peer Review
My Grades
Discussion
Calendar
NOW VIEWING: HOME > THE TAMIL NADU DR.M.G.R.MEDICAL UTY 2014-15 EXAMINATIONS
Class Homepage
This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your first submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.
Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2014-15 Examinations
Info
Dates
Similarity
TNMGRMU EXAMINATIONS
Start 01-Sep-2014 11:27AM
Due 15-Aug-2015 11:59PM
Post 15-Aug-2015 12:00AM
20%
Resubmit
View
Copyright © 1998 – 2014 iParadigms, LLC. All rights reserved.
Usage Policy
Privacy Pledge
Helpdesk
Research Resources
start
Command Wo...
ENGLISH TYP...
THESIS DURA...
FRONT PAGE ...
RAJA PROJE...
Microsoft Exc...
Turnitin - Mozi...
receipt_An as...
4:41 PM

CONTENTS

S.NO	CONTENT	PAGE NO
1.	INTRODUCTION	1
2.	AIM	3
3.	OBJECTIVES	4
4.	REVIEW OF LITERATURE	5
5.	MATERIALS AND METHODS	71
6.	OBSERVATION AND RESULTS	77
7.	DISCUSSION	97
8.	SUMMARY	101
9.	CONCLUSION	103
10.	BIBLIOGRAPHY	
11	ANNEXURE Annexure – I Proforma Annexure – II Master Chart Annexure – III Abbreviations	

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
1.	DISTRIBUTION OF FIBROCYSTIC BREAST DISEASE CASES ACCORDING TO DIFFERENT AGE GROUPS	77
2.	DISTRIBUTION OF MORPHOLOGICAL CHANGES IN FIBROCYSTIC BREAST DISEASE	79
3.	CORRELATION OF EPITHELIAL HYPERPLASIA AND GRADE OF ELASTOSIS	81
4.	CORRELATION OF EPITHELIAL HYPERPLASIA WITH MICRO VESSEL DENSITY SCORE (GRADE)	83
5.	DISTRIBUTION OF INVASIVE DUCTAL CARCINOMA-NOS TYPE CASES ACCORDING TO DIFFERENT AGE GROUPS	85
6.	CORRELATION OF MICRO VESSEL DENSITY GRADE WITH THE AGE OF THE INVASIVE DUCT CARCINOMA-NOS TYPE CASES	87
7.	DISTRIBUTION OF INVASIVE DUCTAL CARCINOMA-NOS TYPE CASES ACCORDING TO HISTOLOGICAL GRADE	89
8.	DISTRIBUTION OF INVASIVE DUCTAL CARCINOMA-NOS TYPE CASES WITH REGARD TO ELASTOSIS GRADE	91
9.	CORRELATION OF HISTOLOGICAL GRADE WITH MICROVESSEL DENSITY GRADE	93
10.	CORRELATION OF HISTOLOGICAL GRADE WITH ELASTOSIS GRADE	95

LIST OF CHARTS

TABLE NO	TITLE	PAGE NO
1.	DISTRIBUTION OF FIBROCYSTIC BREAST DISEASE CASES ACCORDING TO DIFFERENT AGE GROUPS	78
2.	DISTRIBUTION OF MORPHOLOGICAL CHANGES IN FIBROCYSTIC BREAST DISEASE	80
3.	CORRELATION OF EPITHELIAL HYPERPLASIA AND GRADE OF ELASTOSIS	82
4.	CORRELATION OF EPITHELIAL HYPERPLASIA WITH MICRO VESSEL DENSITY SCORE (GRADE)	84
5.	DISTRIBUTION OF INVASIVE DUCTAL CARCINOMA-NOS TYPE CASES ACCORDING TO DIFFERENT AGE GROUPS	86
6.	CORRELATION OF MICRO VESSEL DENSITY GRADE WITH THE AGE OF THE INVASIVE DUCT CARCINOMA-NOS TYPE CASES	88
7.	DISTRIBUTION OF INVASIVE DUCTAL CARCINOMA-NOS TYPE CASES ACCORDING TO HISTOLOGICAL GRADE	90
8.	DISTRIBUTION OF INVASIVE DUCTAL CARCINOMA-NOS TYPE CASES WITH REGARD TO ELASTOSIS GRADE	92
9.	CORRELATION OF HISTOLOGICAL GRADE WITH MICROVESSEL DENSITY GRADE	94
10.	CORRELATION OF HISTOLOGICAL GRADE WITH ELASTOSIS GRADE	96

LIST OF COLOUR PLATES

SI.NO.	TITLE
1.	**FCD WITH DUCTAL EPITHELIAL HYPERPLASIA AND CYST FORMATION (H&E SECTION)**
2.	**FCD WITH APOCRINE METAPLASIA (H&E SECTION)**
3.	**INVASIVE DUCTAL CARCINOMA NOS TYPE (H&E SECTION)-10 X VIEW**
4.	**INVASIVE DUCTAL CARCINOMA NOS TYPE (H&E SECTION) – 40 X VIEW**
5.	**FCD WITH PERIDUCTAL ELASTOSIS (VVG SPECIAL STAIN)**
6.	**IDC-NOS WITH PERIDCTAL AND STROMAL ELASTOSIS (VVG SPECIAL STAIN)**
7.	**FCD – MICRO VESSEL DENSITY CD 34 IMMUNO STAIN – MILD DEGREE (40X)**
8.	**FCD – MICRO VESSEL DENSITY CD 34 IMMUNO STAIN – SEVERE DEGREE (40X)**
9.	**IDC-NOS MICRO VESSEL DENSITY CD 34 IMMUNO STAIN – MILD DEGREE (10X)**
10.	**IDC-NOS MICRO VESSEL DENSITY CD 34 IMMUNO STAIN – MODERATE DEGREE (10X)**
11.	**IDC-NOS MICRO VESSEL DENSITY CD 34 IMMUNO STAIN – SEVERE DEGREE (10X)**

ABSTRACT:

Background:

The tumor progression and metastasis in breast cancer are angiogenesis dependant. Elastosis, the presence of clumps of elastic fibers, is known to occur frequently in association with breast carcinoma.

Aim: To grade angiogenesis and elastosis in Fibrocystic breast disease and Invasive duct breast carcinoma and correlate them with grade .

Material and Methods:

30 cases of fibrocystic breast disease and 30 cases of invasive breast carcinoma were selected and immunohistochemistry with CD34 markers and special stain with verhoff von gieson stain was done for all cases.

Results:

The severe grade of microvessel density is prognostically significant in Fibrocystic disease with epithelial hyperplasia and Invasive duct breast carcinoma - Nos type cases with high grade (gradeIII) histological tumors. The significance of grade of elastosis in Fibrocystic breast disease and Invasive ductal breast carcinoma is obscure.

Conclusions:

The microvessel density may have a role as one of the prognostic factors in node negative ductal breast carcinomas.

Keywords:

Fibrocystic breast disease, Invasive duct breast carcinoma – Nos type Angiogenesis , Microvessel density grade, ,Elastosis grade.

INTRODUCTION

Fibrocystic disease is one of the most common benign condition of the breast. Its incidence is significantly increased by nulliparity, late age of first birth, and late menopause[26], factors also associated with increased risk for breast carcinoma risk. The diet such as high intake of meat fat and caffeine[2] have been associated with a greater risk of proliferative breast disease. Ductal and lobular hyperplasia, cyst formation, adenosis and fibrosis are major morphologic changes of Fibrocystic breast disease.

Ductal hyperplasia is a frequent constituent of fibrocystic change that may be detected by mammography or as a palpable tumor. Moderate or florid hyperplasia without atypia and sclerosing adenosis both are at slightly increased risk of breast carcinoma.[2,4]

Breast cancer is the second most common cancer in women.In 2007 an estimated 178,480 women were diagnosed with invasive breast cancer[12], 62,030 with carcinoma in situ, and over 40,000 women died of the disease. Breast cancer is the second leading cause of cancer related deaths in females all over the world. It has doubled in India in the last

two decades. As per study conducted in Coimbatore, percentage of occurrence of breast cancer was found to 10.5% (159 cases /year).

The angiogenesis associated with breast carcinomas has attracted interest as a prognostic indicator[4]. Pathologic studies of angiogenesis in breast carcinoma have examined the relevance of tumor vascularity to known prognostic markers and to prognosis. To perform such studies histologic sections of paraffin embedded tissue are stained with immunohistochemical markers for endothelial cells such as CD34. In this study the objective is to assess the role of angiogenesis on disease progression from FCD to breast carcinoma.

High microvessel density has been shown in some studies to be associated with a poor grade of histologic differentiation in invasive duct carcinomas and with a greater probability of axillary nodal metastases[27].

Epithelial hyperplasia is a premalignant component of fibrocystic breast disease and shows higher degree of elastosis[114] when compared to other morphologic changes of FCD. The significance and pathogenesis of elastosis in breast carcinoma is unknown. It is common in infiltrating ductal and lobular carcinomas of the breast.

AIM OF STUDY

To assess microvessel density and elastosis in fibrocystic breast disease and invasive breast carcinoma.

OBJECTIVES

1) To grade microvessel density using CD34 immunohistochemical marker in fibrocystic breast disease and invasive duct carcinoma.
2) To grade of elastosis using Verhoffs VanGieson stain in fibrocystic breast disease and invasive duct carcinoma.
3) To correlate grade of elastosis and score (grade) of microvessel density with ductal epithelial hyperplasia in fibrocystic breast disease.
4) To correlate grade of elastosis and score (grade) of microvessel density with histological grade of invasive duct carcinoma.

REVIEW OF LITERATURE

Fibrocystic disease is one of the most common benign condition of the breast. Its incidence is significantly increased by nulliparity, late age of first birth, and late menopause, factors also associated with increased risk for breast carcinoma[27] . The diet such as high intake of meat fat[2] and caffeine have been associated with a greater risk of proliferative breast disease.

The ductal and lobular hyperplasia, cyst formation, adenosis and fibrosis are major morphologic changes of FCD. The ductal hyperplasia is a frequent constituent of fibrocystic changes that may be detected by mammography or as a palpable tumor. Moderate or florid hyperplasia without atypia and sclerosing adenosis both are at slightly increased risk of breast carcinoma.

Breast cancer is the most common site specific cancer in women and is the leading cause of death for women aged 20 to 59 years. High incidence in North America and northern Europe (91.4 new cases per 100 000 women/year), intermediate incidence in southern European and Latin American countries, and low incidence in Asian and African countries[12].

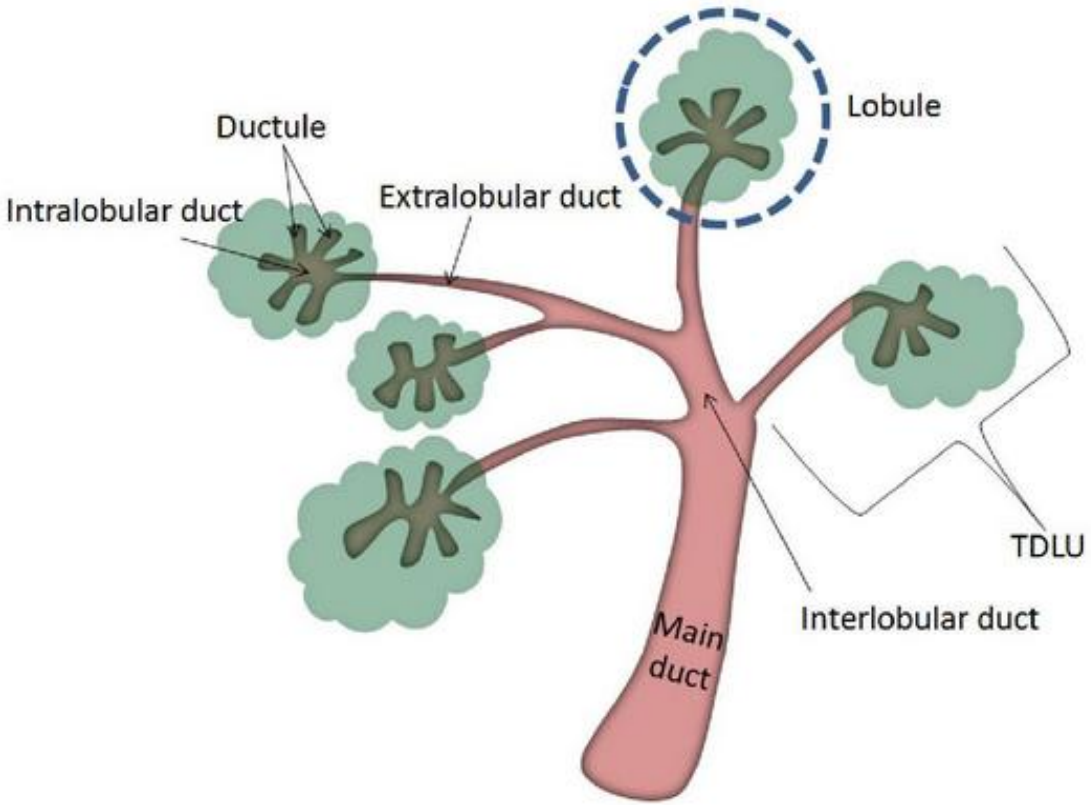
In the United States, there has been a sharp increase in the detection of breast carcinoma, largely due to the widespread use of mammography[20]. Most of these cases have been localized, measuring less than 2 cm in diameter with/without situ carcinoma. The breast cancer mortality rate has (or) ocmedown from 30%to20% because of early screening and more effective treatment.

ANATOMY OF BREAST

The Breast (Mammary gland) is a highly modified apocrine sweat gland, made up of lobules and ducts. The mammary gland is covered with skin and subcutaneous tissue and rests on pectoralis muscle, from which lobules it is separated by a fascia.[2]

The functional unit of breast is composed of two major parts: Terminal duct lobular unit (TDLU) and larger duct system. The TDLU[2] is formed by the lobule and terminal ductule and represents the secretory portion of the duct.

TERMINAL DUCTAL LOBULAR UNIT



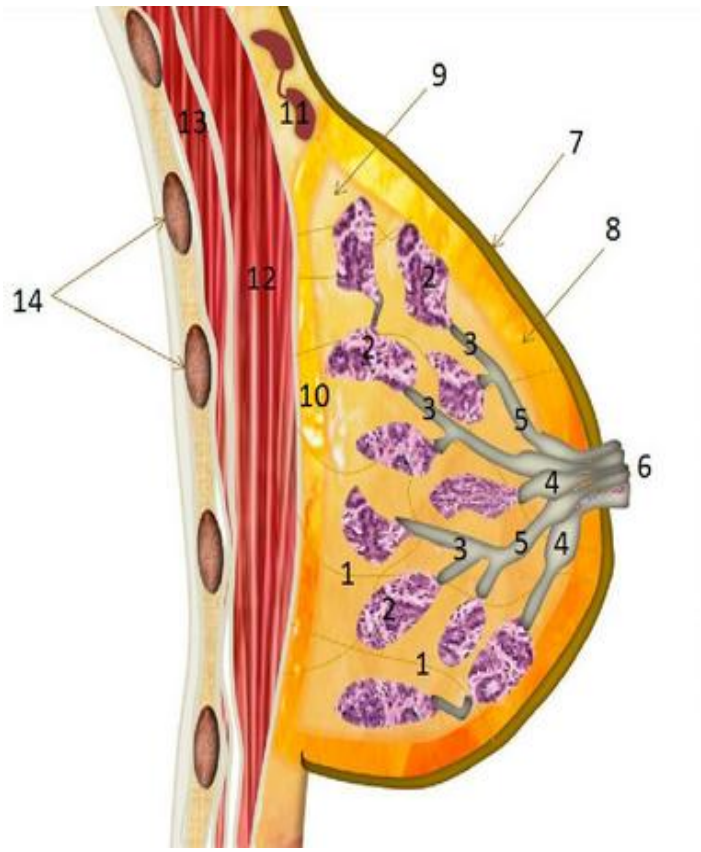
The functional unit of breast is composed of two major parts:

(1) Terminal duct lobular unit (TDLU)

(2) Larger duct system.

The TDLU is formed by the lobule and terminal ductule and represents the secretory portion of the duct.

BREAST ANATOMY

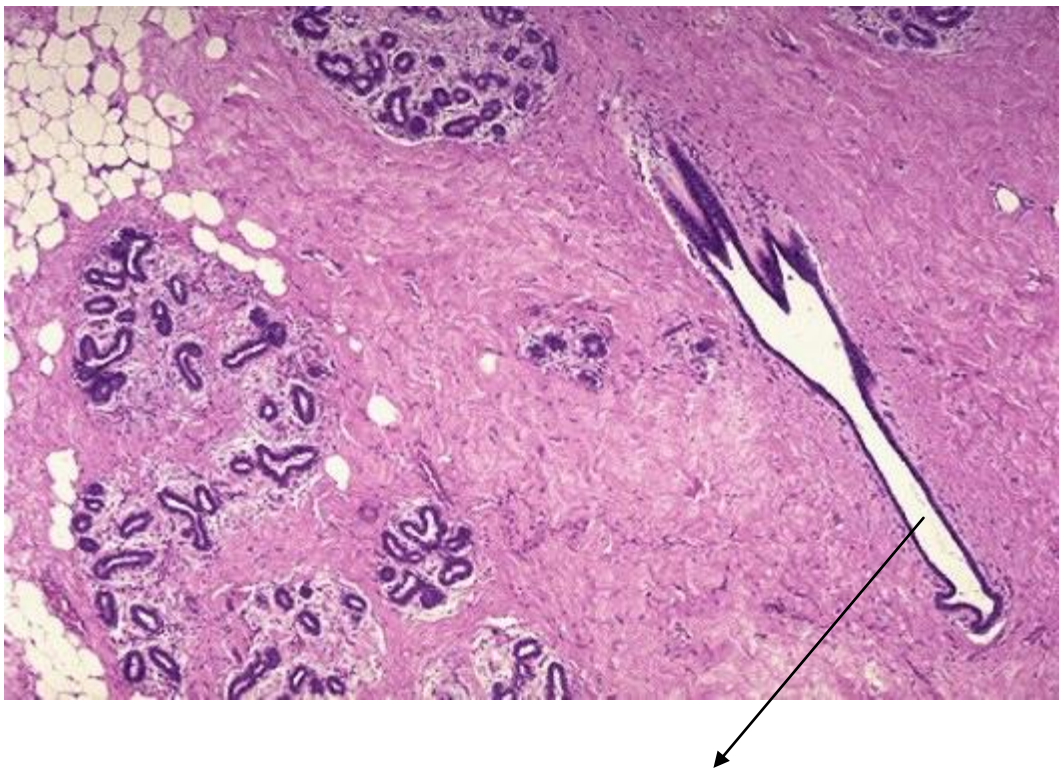


1. Cooper's Ligaments
2. Breast Lobule
3. Extralobular Duct
4. Ductal Ampulla (Reservoir)
5. Main Duct
6. Nipple
7. Skin
8. Subcutaneous Fat
9. Mammary Layer Fatty Tissue
10. Retromammary Fat
11. Lymph nodes
12. Pectoralis Major muscle
13. Pectoralis Minor muscle
14. Rib

HISTOLOGY OF BREAST:

The lobules are separated by less dense collagenous interlobular tissue, whereas the intralobular supporting tissue surrounding the ducts within each lobule is less collagenous and more vascular.

The ducts and lobules are lined by inner secretory epithelial cells and outer myoepithelial cells[1]. Epithelial cells are columnar or cuboidal cells, and myoepithelial cells situated under epithelial cells have smaller, dark nuclei.



H & E Section Shows Terminal ductal lobular unit

FIBROCYSTIC DISEASE OF BREAST:

Fibrocystic breast disease or change is a most common benign lesion of the breast consisting of cystic dilation of intra lobular glands. It occurs between the ages of 25 and 45 years. More than 50 % of women present with illdefined palpable mass, cyclical pain, and tenderness. The exact cause of FCD is not known, but hormones may have role in its development.

Morphologic changes of FCD[2]

1. Cyst formation
2. Apocrine metaplasia
3. Fibrosis
4. Adenosis
5. Calcification
6. Chronic inflammation
7. Epithelial hyperplasia
8. Fibroadenomatoid change.

1. CYSTS:

Grossly multiple cysts are seen and measure between 2mm to lessthan 1 cm in size. Microscopically cysts are lined by a flattened *atrophic* epithelium or by metaplastic apocrine cells[2].

2. FIBROSIS:

It is secondary to rupture of the cysts[2].

3. APOCRINE METAPLASIA:

Apocrine metaplasia[2] is a very common finding in fibrocystic disease. It is mostly seen in dilated and cystic structures. The apocrine cells have abundant eosinophilic ,granular cytoplasm and round vesicular nuclei.

4. ADENOSIS :

Adenosis refers to increase in the number of acini per lobule[12]. The acini are enlarged but not distorted. The acini are lined by benign appearing columnar cells. This lesion is the earliest recognizable precursor of epithelial neoplasia. Sclerosing adenosis is associated with moderately increased risk for breast carcinoma.

OTHER CHANGES :

5. CALCIFICATION:

It is a less common change. Its chemical composition[2] is calcium oxalate, easily identified on mammography and H&E sections.

6. CHRONIC INFLAMMATION:

This results from rupture of cysts[2]. The inflammatory cells are lymphocytes, histiocytes and plasma cells.

7. EPITHELIAL HYPERPLASIA:

It is the most significant[2] pathology of FCD. The epithelial hyperplasia is defined by the presence of more than two cell layers. Microscopically the benign conditions of epithelial hyperplasia is identified by following features,

1) Nuclei are oval, normochromatic and with mild overlap; small, single, indistinct nucleoli; mild or no mitotic figures.

2) The cytoplasm is acidophilic and finely granular [2]and the borders are indistinct.

3) Streaming effect, induced by the oval cells being vaguely arranged in parallel bundles and 'Tufts' and 'mounds' projecting into the lumen.

4) Presence of peripheral elongated clefts, bound on one side by a single layer of basally located cells and on the other by a solid intraluminal formation.

5) Presence of irregularly shaped bridges connecting opposite portions of the wall. The cells in these bridges have oval nuclei arranged parallel to the long axis of the bridge. This is referred as Roman bridges.

6) Complete or incomplete apocrine metaplasia with cytoplasmic blebbing.

7) Presence of myoepithelial cells.

8) Presence of foamy macrophages, both in the lumen and intimately admixed with the proliferating epithelial cells.

9) Stromal Calcification or intraluminal calcification are common.

10) Absence of necrosis.

8. FIBROADENOMATOID CHANGE:

It is rare change of FCD.A study conducted in 1998 by the Cancer Committee of the College of American Pathologists[27]defined the relative risk for breast carcinoma associated with proliferative breast lesions as follows.

NO INCREASED RISK

- Adenosis, other than sclerosing adenosis
- Duct ectasia
- Fibroadenoma lacking complex features
- Fibrosis
- Mastitis
- Hyperplasia without atypia
- Cysts, gross or microscopic
- Simple apocrine metaplasia without associated adenosis
- Squamous metaplasia

SLIGHTLY INCREASED RISK

- Complex fibroadenoma
- Moderate or florid hyperplasia without atypia
- Sclerosing adenosis
- Solitary papilloma without atypical hyperplasia

MODERATELY INCREASED RISK

- Atypical ductal hyperplasia.
- Atypical lobular hyperplasia

Sl.No.	BREAST LESIONS	RELATIVE RISK OF CARCINOMA
1	Non proliferative breast lesion	3%
2	Proliferative breast disease without atypia	5-7%
3	Proliferative breast disease with atypia	13-17%
4	Carcinoma in situ	25-30%

BREAST CARCINOMA:

Breast cancer is the most common site specific cancer in [13]women and is the leading cause of death for women aged 20 to 59 years. High incidence in North America and northern Europe (91.4 new cases per 100 000 women/year), intermediate incidence in southern European and Latin American countries, and low incidence in Asian and African countries[2].

In the United States, there has been a sharp increase in the detection of breast carcinoma, largely due to the widespread use of mammography[13]. Most of these cases have been localized, measuring less than 2 cm in diameter with or without in situ carcinoma. The breast cancer mortality rate has slowly decreased from 30%to20% because of early screening more effective treatment.

CLINICAL FEATURES OF BREAST DISEASE:

1) Pain
2) Palpable mass
3) Lumpness
4) Nipple discharge

RISK FACTORS OF BREAST CANCERS:

1) AGE :

The peak incidence at age of 75 to 80years and rarely occurs in less than 25 years of age. Younger women with breast carcinoma have a worst prognosis than older women[66].

2) AGE AT MENARCHE:

The risk of breast cancer is 20% increased with Women attained menarche at less than 11 years of age. Late menopause also increases the risk of cancer[12].

3) RACE /ETHNICITY:

The risk of breast cancer is highest in Non –hispanic white women. The P53 mutation are more common in African American women but less common in Hispanic women[12].

4) AGE AT FIRST LIVE BIRTH:

The risk of breast cancer is higher in Women with first full-term pregnancy[12] at ages less than 20 years and in women over the age of 35 at their first birth or nulliparouswomen.

5) PREGNANCY:

At the time of pregnancy[69] the changes in stroma that leads to the growth and expansion of lobules facilitate the transition from in situ to invasive carcinoma.

6) FAMILY HISTORY:

The risk is increased in first degree relatives. Only 13% of women with breast cancer have one affected first-degree relative, and only 1% have two or more. The family risk is due to low-risk susceptibility genes and nongenetic factors. The BRCA1 and BRCA2 mutations[2,16,70] are associated with increased risk of breast cancer.

7) HORMONAL FACTORS:

The women taking postmenopausal hormone replacement therapy risk of breast cancer is increased in 1.2 to 1.7 fold. Most of the cancers are ER-positive carcinomas and invasive lobular carcinomas. Oophorectomy may decreasing endogenous estrogens which also reduce the risk of developing breast cancer by up to 75%. Low frequency of fibrocystic disease among long term use of oral contrceptives[29].

8) ATYPICAL HYPERPLASIA AND FCD :

Atypical hyperplasia is associated with increased risk of invasive carcinoma[2]. In FCD (Epithelial hyperplasia without atypia) is associated with moderate risk of breast cancer[2,4].

9) EXPOSURE OF RADIATION:

The radiation especially chest radiation[12] is associated with high risk of developing breast cancer.

10) CONTRALATERAL BREAST CANCER:

The possibilities that a patient with invasive breast cancer will acquire cancer in the contralateral breast [18] is about 5 times that of the general population and also higher in family history of breast carcinoma. The risk increased as 25%-50% in lobular carcinoma.

11) DIET:

The risk is reduced with consumption of Caffeine and heavy consumption of alcohol[12] is associated with increased risk of breast cancer.

12) OBESITY:

The risk of breast cacer is decreased in premenopausal obese women, but the risk high in postmenopausal obese women[12] .

13) BREAST FEEDING:

The risk is decreased in breast feeding women.

14) TUMOR LOCATION:

About 50% of the breast carcinoma is located in upper outer quadrant of breast then 15% in the upper inner quadrant,17%in the central region 10% in the lower outer quadrant and 5% in the lower inner quadrant[2].

15) TOBACCO:

The breast cancer not associated with smoking but that leads to periductal mastitis[12].

16) ENVIRONMENTAL TOXINS:

Organochloride pesticide is associated with increased risk of breast carcinoma.[12]

ETIOLOGY AND PATHOGENESIS:

The Genetic[16] and Hormonal factors[30] are the main risk factors for the growth of breast carcinoma. The breast cancer is divided in to sporadic breast cancer and hereditary breast cancer.

The hereditary gene is a primary source of the breast carcinoma. The possibility of a hereditary etiology increases with variable affected 1st-degree relatives.

The BRCA1 and BRCA2 mutation related greater part of breast cancers, attributable to single mutations and about 3% of all breast cancers.

Mutations in BRCA1 also manifestly raise the risk of rising ovarian cancer but mutation in BRCA2 confers a less risk for ovarian cancer and linked more commonly with male breast carcinoma.[12]

Mutation in BRCA1-related breast carcinomas are usually poorly differentiated tumor and do not express hormone receptors (Triple negative cancer). The BRCA1 carcinomas are often linked with loss of the inactive X chromosome, resulting in the lack of the Barr body.

MOLECULAR GENETICS OF BREAST CANCER:

1. Growth receptor overexpression
2. Growth factor overexpression
3. Intracellular signaling molecule alterations
4. Cellcycle regulator alterations

5. Adhesion molecule alterations
6. CMYC amplification

MOLECULAR SUSTYPES:

1. 1.Luminal type (type A , B and C)
2. HER2/neu type
3. Normal breast –like

Luminal A type -50% of invasive breast carcinoma, ER/PR positive and HER2/neu negative. Good prognosis[2].

Luminal B type- 20% of invasive breast carcinoma, ER/PR positive and variable expression of HER2/neu receptor. Respond to endocrine treatment and less prognosis.

HER2/neu - 15% of invasive breast carcinoma,ER/PR negative and HER2/neu positive and respond to trastuzumab with poor prognosis.

Basal –like - 15% of invasive breast carcinoma. Majority of ER/PR and HER2/neu receptors are negative and no respond to trastuzumab or endocrine treatment with poor prognosis[2]. Basal-like types is extremely heterogenous, and encompasses a few tumors with good

prognosis, such as secretory carcinoma, medullary carcinoma and adenoid cystic carcinoma.

SPORADIC BREAST CARCINOMA :

The main risk factors for sporadic cancer[12]are associated with hormone introduction, sex, age at menarche and menopause, reproductive record, breast feeding, and exogenous estrogens. The majorities of sporadic carcinoma arise in postmenopausal women and are positive estrogen receptors.

INVESTIGATIONS:

1) CLINICAL EXAMINATIONS:

It is age old method for the detection and evaluation of breast disease. It als most useful method[2] for physician and patient herself. The clinical impressions are incorrect in 15% of benign cases and 10% of cases of malignant.

Axillary lymph node examination is also important. The positive nodes will be found free of metastases microscopically in 15% of cases.

2) MAMMOGRAPHY:

The widespread use of mammography[20] has a important diagnostic method of breast cancer. The small tumors (1–2 mm) can be identified by mammography. It usually appears on mammography on the presence of calcification. The incidence of calcification associated with breast carcinoma is about 50-60%[2,4].

The negative mammogram does not rule out the possibility of the presence of carcinoma, since about twenty percent of palpable tumors are not detectable with this technique and false positive incidence is 1%.

The proper handling of breast lesions detected by mammography requires team work of radiologist, surgeon, and pathologist. The Abnormal area identified on mammography, provides the surgeon with a doubtful area within the breast.

After excision of specimen lateral margins should be marked by sutures and an x-ray study taken of the specimen. If no lesion is seen, the surgeon should obtain additional tissue.

3) MRI IMAGING:

The nuclear magnetic resonance with contrast-enhanced techniques are more informative and most useful method of breast carcinoma. It is also more sensitive for the detection of multicentric breast cancer.

4) ULTRASONOGRAPHY:

It is particularly for detection of cystic or solid lesions.

5) CYTOLOGY:

The two methods are used to obtain cytologic material from breast lesions are

(1) Aspiration of nipple secretion

(2) Fine needle aspiration cytology (FNAC).

The cytology of nipple secretion is now limited useful method for breast lesions. FNAC[21] is indicated in all palpable breast lesions. It is a rapid, accurate and cost effective method of diagnosis. The sensitivity is about 87%, the specificity is about 100%[2,13]. The adequacy of breast FNAC is about six clusters of epithelial cells spread over two glass slides.

The most of benign lesions of breast misdiagnosed cytologically as malignant lesions such as the fibrocystic breast disease with marked epithelial proliferation[2,4]. On cytologically distinguish between in situ and invasive ductal carcinoma is not possible. The histologic sections prepared from cell blocks of aspirated materials may help in identification of invasion.

The role of FNAC

1) To diagnosis of simple cyst
1) The preoperative confirmation of clinically suspected cancer
2) To investigate of suspected recurrence or metastasis in case of previously diagnosed cancer.
3) To obtain tumor cells for special analysis such as IHC and DNA analysis.

6) BIOPSY:

The needle core biopsy[2,22] is a nonoperative diagnosis of breast cancer. This is based on the both cytologic and architectural features, and gives a correct diagnosis of invasive breast carcinoma and a benign breast lesions. It also helps to identify microcalcifications.

In addition, prognostic indicators (ER, PR, HER2/neu, etc.) can be evaluated in core needle biopsies. Accurate diagnosis of a core biopsy is about >90% .

A) OPEN BIOPSY:

It is two types

1) Excisional biopsy
2) Incisional biopsy

The excisional biopsy usually performed in when the tumor measures 2.5 cm or less and the incisional biopsy for larger tumors. This technique is highly accurate and the false positive rate is 0 %, the false negative rate is<1 %.

B) FROZEN SECTION:

It is most useful method for the confirmation of a cytologic diagnosis breast carcinoma or the evaluation of surgical margins[2,23].

Disadvantages: Difficulties with frozen sections are usually attributable to

(1) Sampling errors

(2) Technical errors

(3) Histologic misinterpretation.

The intraoperative cytologic examination can be taken simultaneously with the frozen section procedure.

7) SENTINAL NODE BIOPSY:

It is based on the sentinel node[58,59] is negative, the other nodes also be negative. But the sentinel node is positive the changes of other nodal metastasis is about one third cases.

The tumor size and the presence of lymphovascular invasion most predictors of positive sentinel nodes. A focal metastasis in a sentinel node is mostly located in the region of the inflow junction of the afferent lymph vessel than other sites.

8) ANCILLARY STUDIES:

To assess the estrogen and progesterone receptor status is a important prognostic indicater and also used to the treatment choice in patients with breast cancer.

The ER and PR receptors[2,4] are measured by immuno histochemical method. The majority of breast carcinomas are ER positive(80%).

SPREAD AND METASTASES:

The breast cancer spread by

1. Direct invasion
2. Lymphatic spread
3. Blood vessel route.
4. Local invasion
5. The distant metastasis to other organs.

The stromal invasion [2,9]can be by direct extension or intramammary lymph vessels, and also by the tissue spaces are known as 'pseudoangiomatous stromal hyperplasia'. Local recurrence after mastectomy appears as superficial nodules.

The most common sites for distant metastasis of breast cancer is lung, pleura, liver, ovary, adrenal gland and central nervous system. Invasive lobular carcinoma is associated with metastasis to the gastrointestinal tract, ovaries and serosal surfaces[2,4].

The lymph node of axillary group and internal mammary lymph nodes are usually involved with metastatic breast carcinoma. \

The supraclavicular lymph node involvement is less commonly involved[2;11]. The incidence of metastatic involvement of the breast carcinoma is approximately 22%.

TREATMENT:

The treatment of breast carcinoma is depends on the type and extent of the tumor.

1) Surgery

2) Radiation therapy

3) Hormonal therapy

4) Chemotherapy

5) Target therapy

The surgical therapy are Halsted's radical mastectomy, which include partial mastectomy and total (simple) mastectomy[6].

Radiation therapy is employed in a postoperative adjunct and for the control of local recurrence of disease.

The chemotherapy [2,4]is used as an adjunct therapy after local treatment and also used in patients with positive axillary nodes.

Chemotherapy has also been used in combination with conservative surgery and radiation in patients with localized large (>3 cm) tumors in order to avoid mastectomy.

The hormonal therapy is used in all stages of estrogen receptor-positive breast cancer[2,4]

WHO CLASSIFICATION[73] OF BREAST TUMORS:

A) EPITHELIAL TUMORS:

Invasive duct carcinoma, Not otherwise specified

Invasive Lobular carcinoma

Tubular carcinoma

Invasive Cribriform carcinoma

Invasive Papillary carcinoma

Invasive Micropapillary carcinoma

Medullary carcinoma

Mucinous carcinoma

Apocrine carcinoma

Metaplastic carcinoma

Secretory carcinoma

Neuro Endocrine carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Micro invasive carcinoma

Intraductal proliferative lesions

- Usual ductal hyperplasia
- Flat ductal hyperplasia
- Atypical ductal hyperplasia
- Ductal carcinoma in situ

Intraductal papillary neoplasm

-Central papilloma

-peripheral papilloma

-Intraductal papillary carcinoma

Benign epithelial proliferation

-Adenosis and its variant

-Radial scar or complex sclerosing lesion

-Adenomas and its variants

B) MYOEPITHELIAL LESIONS:

- Myoepitheliosis
- Adenomyoepithelial adenosis
- Adenomyoepithelioma
- Malignant myoepithelioma

B) MESENCHYMAL TUMORS:

- Hemangioma
-Angiomatosis
- Hemangiopericytoma
- Myofibroblastoma
- Fibromatosis
- Inflammatory myofibroblastoma
- Lipoma, Leomyoma
- Granular cell tumor
- Neurofibroma
- Schwannoma
- Angiosarcoma, Liposarcoma, Rhabdomyosarcoma, Osteosarcoma And Leomyosarcoma

D) FIBROEPITHELIAL TUMORS:

- Fibroadenoma
- Phylloides tumor
- Periductal stromal sarcoma
- Mammary hamartoma

E) TUMORS OF NIPPLE:

- Nipple adenoma
- Paget disease of nipple

F) MALIGNANT LYMPHOMA:

- Diffuse Large B cell Lymphoma
- Burkitts Lymphoma
- Extra nodal marginal zone B cell Lymphoma of MALT type
- Follicular Lymphoma

G) METASTATIC TUMORS

H) TUMORS OF MALE BREAST:

- Gynaecomastia

-Carcinoma- nvasive and insitu

DUCTAL CARCINOMA IN SITU (DCIS):

DCIS is also refered as intraductal carcinoma[2] of the breast. It consists of a malignant clonal population of cells limited to ducts and lobules by the basement membrane[94]. The myoepithelial cells are preserved.

Morphologic variants:

1) Comedocarcinoma

2) Solid variant

3) Cribriform variant

4) Papillary variant

5) Micropapillary variant

6) Clinging variant

7) Cystic hypersecretory variant.

COMEDOCARCINOMA[94]:

Grossly it shows a cluster of thick walled ducts with normal breast parenchyma. The evidence of necrosis is present.

Microscopically tumor cells are arranged in solid sheets. The tumor cells exhibit high degree of hyperchromasia and pleomorphism.Necrosis and mitosis is present. The myoepithelial cells surrounds the duct. The stroma shows periductal fibrosis, infiltration of mononuclear cells[2,9].

SOLID VARIANT:

The proliferating cells completely fill within the gllndular lumen.

CRIBRIFORM VARIANT:

In this lesion intraepithelial spaces are evenly distributed and are regular in shape.

PAPILLARY VARIANT:

The papillary variant arises fom large ducts. Grossly it a well defined mass.Microscopically the tumor cells are arranged in papillary[95] paterrn with fibrovascular core.The tumor cells show,loss of nuclear polarity ,marked hyperplasia and lack of myoepithelial cells.

DUCTAL INTRA EPITHELIAL NEOPLASIA(DIN):

1a-related to usual ductal hyperplasia, 1b-related to flat epithelial atypia, 1c-related to ADH and small grade IDCIS, 2-related to large grade I DCIS and grade II DCIS, 3-related to grade III DCIS.

CLASSIFICATION OF DCIS:

A. Lagios classified by

-High grade DCIS

-Intermediate grade DCIS

-Low grade DCIS

B. Nottingham classified by

-Pure comedo DCIS

-DCIS with necrosis

- DCIS without necrosis

C. Van nuys classified by

-High grade DCIS

-Non high grade DCIS with necrosis

-Non high grade DCIS without necrosis

D. Holland and Hendricks classified

-Poorly differentiated DCIS

-Intermediately differentiated DCIS

-Well differentiated DCIS

E. NHSBSP/EEC classified

-High grade DCIS

-Intermediate grade and low grade DCIS

DCIS MICROSCOPIC FEATURES:

1. Luminal bridges and intercellular spaces appear rigid.
2. Presence of necrosis.
3. Evenly distributed cells
4. Monomorphic population of tumor cells
5. Apocrine metaplastic foci are not seen
6. Streaming of nuclei not seen

7. Minimal nuclear pleomorphism and nuclear hyperchromasia ,prominent nucleoli are present. The mitotic figures are inconsistent.

PROGNOSTIC INDEX OF DCIS:(VANNUYS PROGNOSTIC INDEX)

1. **Lesion size**

   Score1- ≤ 15mm

   Score - 16 - 40mm

   Score 3 - > 40mm

2. **Margin**

   Score1-. >10mm

   Score2 -1to 9mm

   Score 3- <1mm

3. **Histology**

   Score1-. Non high grade without necrosis

   Score2 - Non high grade with necrosis

   Score 3- High grade

AJCC STAGING OF BREAST CARCINOMA

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	AnyM	

INVASIVE DUCTAL CARCINOMA NOS TYPE:

Invasive ductal carcinoma are malignant tumors of the breast which have the tendency to invade the adjacent normal breast tissues and also the distant organs[2,4]. They can be classified into various histological types. Invasive duct carcinoma not otherwise specified (NOS) is the most common type. Others names used are, Ductal carcinoma, Carcinoma not otherwise specified and invasive carcinoma of no special type. According to WHO it is called Invasive duct carcinoma ,no special type, and it about 75-80% of breast carcinoma.

The chief presenting complaint in carcinoma breast is palpable lump. The size of tumor is variable from 1 cm to several centimeters in diameter. Cut surfaces are firm to hard and the margins are well or poorly defined. About 2 percent of the patients present with the synchronous bilateral tumors. Pain and nipple discharge is rare presenting complaint of breast carcinoma.

The mammographic evaluation of breast screening has changed the pattern of presentation .With the help of mammographic screening most of the patients with breast carcinomas detect impalpable mammographic abnormality.

The mucinous (colloid) carcinoma, tubular carcinoma, papillary carcinomas, medullary carcinoma, secretory carcinoma, and adenoid cystic carcinoma are breast carcinomas with favourable prognosis. Where as metaplastic carcinoma, pleomorphic lobular carcinoma and inflammatory carcinoma have unfavourable prognosis.

As per the American Cancer Society[6], about two-thirds of women with an invasive duct carcinoma NOS are 55years or older. The overall survival rate is 55-65%.

HISTOLOGICAL SPECTRUM OF INVASIVE BREAST CANCER

1) Invasive ductal carcinoma –NOS type	-	70-89%
2) Invasive lobular carcinoma	-	10%
3) Tubular / Cribriform carcinoma	-	06%
4) Medullary carcinoma	-	02%
5) Mucinous carcinoma	-	02%
6) Papillary carcinoma	-	01%
7) Metaplastic carcinoma	-	< 01%
8) Others	-	13%

INVASIVE DUCT CARCINOMA NOS TYPE:

Histopathology:

Gross:

It is poorly circumscribed, firm to hard, gritty to cut, with chalky white streaks. The size may vary from 0.5-10cm.

Microscopy:

Tumor cells are arranged in diffuse sheets, well defined nest, or as individual cells. The nuclei are regular or large pleomorphic with prominent nucleoli. Depending on the grade of the tumor the mitotic figures are varible.

The stroma are densely fibrotic[2,4] to highly cellular. Areas of focal necrosis, squamous metaplasia and apocrine metaplasia or clear cell changes are seen. Over 90% of cases associated with elastosis. In between tumor and sroma shows variable amounts of mononuclear inflammatory infiltration.

According to study conducted by Fisher et al the percentage of perineurial, lymph vessel and blood vessels invasion are 28%,33% and 5% of cases respectively.

OTHER SPECIAL TYPES:

TUBULAR CARCINOMA:

The incidence of tubular carcinoma[34] is 1-3 %.Tubular carcinoma is a well differentiated tumor and have an good prognosis.

Gross:

Tubular carcinoma is usually small, and about 1 cm in diameter. Cut surface shows ill defined firm to hard greyish white tumor usually lessthan 2cm in size.

Histopathology:

Microscopically it is characterized by haphazardly arranged irregular angulated tubules witen lumen with basophilic secretion.The lumen lined by only one layer of epithelial cells with mild nuclear pleomorphism and rare mitosis. The stroma is abundant and cellular. Periphery of the lesion shows invasion of fat also seen.

CRIBRIFORM CARCINOMA:

It's rare form of invasive carcinoma with an excellent prognosis[35]. The incidence is >3%.

Gross:

solid greyish white in appearance.

Histopathology:

Tumor cells arranged in cribriform pattern. Tumor cells are small cells and exhibit mild or moderate degree of pleomorphism and rare mitosis. Stromal invasion may be present.

MUCINOUS CARCINOMA:

The incidence of the tumor is 1-4%.It also known as colloid carcinoma[36]. These are tumors in which mucin production is apparent to naked eye. It usually occurs in postmenopausal women and has a favorable prognosis.

Gross:

Well defined mass with a soft and gelatinous consistency. Areas of hemorrhages are more common.

Histopathology:

Small clusters of regular epithelial cells floating in a pool of extracellular mucin .The tumor cells are small with darkly staining nuclei which exhibit mild pleomorphism. Mitoses are rare.

MEDULLARY CARCINOMA:

The frequency varies between 2-10 % and common in Japanese women and mostly associated with BRCA1 mutations.

Gross:

Well circumscribed large tumor with pushing margins and soft consistency.

Histopathology:

Three main morphologic criteria for diagnosis of medullary carcinoma[2,37]

1. Tumor cells are arranged in Syncytial pattern of growth. The malignant cell are pleomorphic nuclei and nucleoli are prominent.
2. The stroma shows lymphoplasmacytic infiltration.

INVASIVE PAPILLARY CARCINOMA:

It constitutes <1% of cases. It usually occurs in elderly. It is entirely insitu lesions[38].

Gross:

Most of the tumors are well demarcated mass measures 1to 3cm, and soft in consistency.

Histopathology :

The malignant cells are arranged in papillary growth pattern. The nuclear atypia is mild to moderate. PapillaryCIS present in more than 70% of cases.

INVASIVE MICROPAPILLARY CARCINOMA:

Rare variant of infiltrative carcinoma with poor prognosis.[2,4,39]

Gross: It is expansive growth and lobulated appearance.

Histopathology:

Cluster of tumor cells arranged in a micropapillary or tubular-alveolar pattern without a fibrovascular core . This tumor is usually associated with high incidence of vascular invasion[40] and lymph node involvement .Over 50% of cases associated with psammoma bodies.

SECRETORY CARCINOMA :

It is rare tumors occurs usually in childhood, but also occur in adolescence .

Gross:

Well circumscribed tumor mass, usually small in size (less than 2 cm in diameter).

Histopathology:

It composed of three histologic pattern[41]

(1) Microcystic pattern composed of small cysts.

(2) Compact solid pattern.

(3) Tubular pattern which contains numerous tubular spaces and containing secretions. Tumor cells have abundant pale eosinophilic cytoplasm with mild pleomorphic nuclei.

Tumor shows a well defined border with peripheral fibrosis. The diagnostic feature is the presence of intra and extracellular rounded vacuoles giving a overall clear cell pattern. The mitosis is infrequent. These vacuoles positive stain for Alcian blue and PAS with diastase resistant.

APOCRINE CARCINOMA:

Its rare type of invasive duct carcinoma[40] with prominent apocrine differentiation. The incidence is 1 to 4%.

Gross:

Cut surface shows brownish –tan colour

Histopathology:

The tumor cells are arranged cords, tubules and solid pattern[2,4]. The malignant cells are large cells have abuntant eosinophilic granular cytoplasm with vesicular hyperchromatic nuclei,and prominent nucleoli.Apocrine snout is seen in luminal portion of tumor.

METAPLASTIC CARCINOMA:

Metaplastic carcinoma[42] rare malignant tumor with admixture of (adeno)carcinoma and spindle cells, squamous cells, chondroid or osseous changes also occur.

Gross:

Large , nodular tumors usually greater than 5cm in diameter and fixed to deep fascia.

Histopathology:

Malignant epithelial elements show transtition to sarcomatous elements which includes cartilage, bone, myxoid stroma and spindle cell stroma[2,4].It is a highly malignant tumors with early recurrence and low survival rate.

Types are

(1) sarcomatoid carcinoma

(2) Spindle cell carcinoma

(3) Carcinoma with osteoclast –like giant cells

(4) squamous cell carcinoma

(5) melanocytic differentiation, choriocarcinomatous type, and pleomorphic carcinoma.

CARCINOMA WITH NEUROENDOCRINE DIFFERENTIATION:

It also known as Carcinoid tumor. Bilateral locations and multicenticity can occur[43].

Gross: Usually well circumscribed growth.

Histopathology:

Tumor is composed of epithelial and spindle cells. The tumor cells are small cells and arranged in solid nests ,separated by fibrous tissue.The tumor cells show a "salt-and-pepper" chromatin pattern and fine granular, eosinophilic cytoplasm. Intracytoplasmic mucin also seen.

OTHER RARE TUMORS:

Squamous cell carcinoma, Clear cell carcinoma, Lipid rich carcinoma, Inflammatory carcinoma, Adenoid cystic carcinoma, Acinic cell carcinoma and metastatic carcinoma.

LOBULAR CARCINOMA :

Gross:

Firm to hard mass and tumor borders are irregular and formation of many small hard nodules recembling sclerosing adenosis[2,4].

Histopathology:

Classic variant: Small uniform cells growing in a single file and in a concentric fashion around the lobules. The stroma is abundant and dense fibrous and containing foci of periductal and privenous elastosis[2,4]. The individual cells are small round to ovoid with little cytoplasm and eccentrically placed nucleus exhibiting mild pleomorphism and infrequent mitoses and Lymphocytic infiltration also present.

Other variants include:

Alveolar variant:

Cells similar to classic type arranged in aggregates of 20 or more cells.

Solid variant:

Tumor cells arranged in diffuse sheets and the stroma is scanty.

Tubulolobular variant:

Cells arranged in tubules and cords . These tubules are small in amounts.

Pleomorphic variant:

Classical lobular infiltrative pattern with cells exhibiting

cellular atypia and nuclear pleomorphism[45]. These cells have more eosinophilic cytoplasm.

Histiocytoid carcinoma:[46]

Tumor arranged in diffuse growth pattern. The malignant cells have abundant granular ,foamy cytoplasm.

Signet ring carcinoma:

Malignant cells show intracytoplasmic mucin accumulation, resulting in signet ring appearance.

MIXED TYPES:

The mixed ductal and lobular comprises of tumors in which the ductal component constitutes between 10 and 90 percent of the tumor.

The mixed ductal and special type include any tumors composed of mixture of tubule-lobular, tubular,cribriform or mucinous carcinoma with ductal NOS in which the later forms over 10 percent of the tumor.

UNDETERMINED CARCINOMA:

It accounts 3-4% of invasive breast carcinoma.

MICROINVASIVE CARCINOMA:

Microinvasive carcinoma is defined as any carcinoma in situ of the breast showing one or more areas of stromal invasion not surpassing 1mm in thickness.

SPREAD RELATED BREAT CANCER:

1. INFLAMMATORY BREAST CARCINOMA:

The inflammatory breast carcinoma refers to the whole breast is red and warm, with extensive edema of the skin[2,4], thus resembling the look of mastitis. Clinically presenting inflammatory breast carcinoma may be microscopically free from invasion into the dermis and visc versa.

2 .PAGET DISEASE:

Paget disease is seen in almost in underlying breast carcinoma of in situ ductal type, with or without invasion of stroma.Clinically it is eczema like lesion.

Histopathologically tumor cells are large clear cells with atypical nuclei usually concentrated beside the basal layer of the epidermis but also penetrating the malpighian layer.

PROGNOSTIC FACTORS OF INVASIVE DUCTAL CARCINOMA OF BREAST:

Breast carcinoma is a heterogeneous disease clinically, pathologically, radiologically and differs in its biological potential.

1) AGE : Women <50years-best prognosis

>50years –bad prognosis

<35years– bad prognosis[61], higher risk of recurrence and distant metastasis.

2) TUMOR SIZE:

The size determinator has a greater prognostic significance when measured microscopically than grossly. Tumor size and lymph node status are two independent prognostic variables[76,77].

Saigo and Rosen studied 111 patients with invasive breast carcinoma of 1 cm or less in diameter associated with negative nodes who were treated with a minimum of a modified radical mastectomy and followed for at least 10 years: 75% were alive with no evidence of disease, 4% were alive with recurrent carcinoma, 6% had died of disease, and 15% had died of other causes.

3) STATUS OF LYMPH NODE:

Axillary lymph node status is an important indicator of systemic adjuvant therapy.lt is based on the histological examination of excised nodes rather than clinical examination. Nodal metastasis are divided into three categories based on size .Numerous studies have shown that patients with positive loco-regional nodes have poor prognosis when compared to those without nodal involvement[78,79] .

Lymph node metastasis and prognosis also depends on the number and level of regional nodes involved..The prognosis is worst in more number of nodes involved.

4) TUMOR SITE:

No relationship, but recent study medial location of the tumor was associated 50% risk of systemic relapse and tumor death than lateral location.

5) TUMOR CONFIGURATION:

Tumors with well defined margins have a better prognosis than tumors with infiltrative margins. Infiltrative tumors tend to be larger and have associated with axillary lymph node metastasis. Tumors with stellate configuration with focal necrosis are associated with worst prognosis[80,81].

6) HISTOLOGIC TYPE:

Histologic type with excellent prognosis -Tubular, cribriform, mucinous and tubulolobular carcinoma.

Histologic type with good prognosis: Mixed- ductal NOS and special type and classic lobular carcinoma.

Histologic type with average prognosis: Mixed lobular, medullary and atypical medullary carcinoma.

Histologic type with poor prognosis: ductal NOS,mixed ductal and lobular, solid lobular carcinoma and grade 3 basal type carcinoma.

7) LYMPHOVASCULAR INVASION:

1. Tumor emboli in clear spaces with endothelial lining.

2. Tumor emboli do not assume the same shape as the lymphovascular vessel.

Lymphovascular invasion is specifically of prognostic value in node negative disease and predicts local recurrence after breast conservation surgery and flap recurrence following mastectomy[82].

8) MICROSCOPIC GRADE:

The important part of oncologic pathology has been the identification that the morphological appearance of tumors can be correlated with the grade of malignancy.[83]

In 1957 Bloom and Richardson developed the grading system in the form of numerical scoring system.In 1968 Patey and Scarff method along with Bloom Richardson method has become modified Bloom Richardson grading Method[83]. In 1982 Elston modified the Scarff Bloom Richardson method to improve the assessment of mitotic figures.

In 1991 Nottingham improved the objectivity of histological grading for assessment of each component factor.Leslie et states that histologic grading of invasive breast carcinoma is a valiable prognostic feature. The histologic grading system is a simple and cost effective method which serves to evaluate morphological characteristics semiquantitatively.

Jean. F. Simpson et al states that the Nittinghamm Combined Histologic Grade (NCHG) system is a modification of Scarff-Bloom-Richardson grading system, which combines the analysis of glandular differentiation, nuclear grade, and mitotic activity.[84]

Histologic grading system should be done in formalin fixed paraffin embedded tissue sections.The assessment of mitotic figures affected by delayed formalin fixation.The thickness of section is 4 to 5 microns and staining method is H&E staining method.[83]

Pure tubular, Mucinous and cribriform carcinomas were graded as grade1. Medullary carcinoma was placed under grade 3. Invasive lobular carcinomas were also graded using this method. Pleomorphic variant of lobular carcinoma were graded as grade 3. For mixed carcinoma, grading proved to be a better prognostic factor than typing alone [85].

The most widely accepted Nottingham modification of Scarff Bloom Richardson grading system was used. They include

- Tubule formation
- Nuclear pleomorphism
- Mitotic count

9) TUBULE FORMATION:

Tubular structures are those with central lumen surrounded by polarized tumor cells. Solid clusters with reversal of polarity seen in micropapillary carcinoma should be scored 3 for tubule formation. All

areas of the tumor should be scanned and the proportion of the tumor occupied by definitive tubular structures with central luminal space is assessed.

1 point : Tubular formations in >75% of the tumor

2 point : Tubular formations in 10-75% of the tumor

3 point : Tubular formations in <10% of the tumor

10) NUCLEAR PLEOMORPHISM:

Assessment of nuclear pleomorphism is the subjective element of histological grade.Fritz Rank et al states that criteria for nuclear pleomorphism includes irregularity in size, shape and staining of nuclei[86].The tumor areas having cells with greatest atypia should be evaluated.

1 Point: Nuclei with minimal variation in size and shape.

2 Point: Nuclei with moderate variation in size and shape.

3 Point: Nuclei with marked variation in size and shape.

11) TUMOR MITOTIC COUNT:

It is most prognostically significant of the grading system.

Morphologic criteria for mitotic figures:

1) Absent nuclear membrane
2) Clear hairy extention of the nuclear material, either clotted in a plane (metaphase/ anaphase) or in separate clots.(Telephase).
3) The surrounding cytoplasm should not be eosinophilic.

Two parallel clearly separate chromosome clots should be counted as separate mitosis. Spiky or triangular nucleus with eosinophilic cytoplasm favours apoptosis. Mitotic score depends on the number of mitosis per high power field. The size of the high power field should be standardized.

According to Bloom- Richardson grading system the mitotic figures are to be counted only at the periphery of the tumor.

The mitotic counting begin in the most mitotically active area. Atleast 10 high power field be counted in the same area.

Kupio and collan established substantial variation of Bloom Richardson grading system depending on the mitotic rate and field size[87]. A minimum of 10 field at the periphery of tumor to counted. The mitotic figure should be counted when one notices atleast one

mitotic figure and then proceed to ten consecutive non overlapping fields.

3 to 5 points- Well differentiated tumor (Grade I)

6 to 7 points- Moderately differentiated tumor (Grade II)

8 to 9 point- Poorly differentiated tumor (Grade III)

OTHER PROGNOSTIC FACTORS:

NECROSIS:

Extensive tumor necrosis is associated with high incidence of lymph node metastasis and survival rates also decreased.

TYPE OF MARGINS:

Tumors with pushing margins are associated with good prognosis than with infiltrative margins.

MICROVESSEL DENSITY:

Invasive carcinoma having a prominent vascular component in the surrounding stroma are more aggressive .High grade tumors are associated with high score of MVD.

ELASTOSIS:

The pathogenesis and significance of elastosis in breast carcinoma to be quite obscure.It is increased in invasive carcinoma but

more prominent in lobular carcinoma.Tumors lacking elastosis have showed lower rate of response to endocrine therapy[115].

STROMAL REACTION:

Tumors with a lack of inflammatory reaction at the periphery have a lesser degree of nodal metastases and presumably a better prognosis.

FIBROTIC FOCI:

Presence of scar like area in centre of tumor is an unfavourable prognosis[2].

SKIN AND NIPPLE INVASION:

Presence of skin invasion correlates with decreased survival rate.The tumor invasion of nipple is related with high incidence of axillary metastasis [2].

LYMPHATIC VESSEL AND PERINEURAL INVASION:

This is associated with increased risk of recurrence.

ER/PR RECEPTORS:

The recent use of immunohistochemistry is to identify nuclear hormone receptors, a result that is correlated with a

superior outcome and is an chief interpreter of response to hormonal therapy. ER-positive breast carcinomas show better response to hormonal and chemotherapy.[2,4,12]

HER2/neu RECEPTOR:

The lower survival rate is related with over expression of Her2/neu receptors[2].

RESPONSE TO NEOADJUVANT THARAPY:

The majority of patients complete their surgery and consequently receive systemic treatment. Neoadjuvant therapy is an another method where the patient is treated prior to surgery.The fact that the tumor responds to chemotherapy is a strong favourable prognostic factor[6].

ANGIOGENESIS:

Tumor angiogenesis refer to the growth of new blood vessel toward and within the tumor.It plays a central role in both local tumor growth and distant metastasis in breast carcinoma.In normal life, angiogenesis has a vital role in reproduction, embrogenesis, menstruation and healing and repair. It was first described by Folkman et al[72] in 1971.

Tumor angiogenesis as well as role of measuring intra tumoral microvessel density are independent prognostic factor in both fibro cystic diseases and breast cancer[71].The college of American pathologist has stated thst the study of quantification of tumor angiogenesis is prognostic value in breast carcinoma.

Tumor angiogenesis is known to be regulated by growth factors secreted by host and tumor cells.Solid tumors require neovascularization to grow beyond about $1mm^3$.

Normally the extra cellular matrix and basement membrane is degraded by matrix metalloproteinase.There are inhibitors of metalloproteinase which are present in tissue.The balance between metalloproteinase and their inhibitors are regulated normally.

With increasing histologic grade and transformation from benign to malignant condition,the expression of matrix metalloproteinase is increased.There is increased level of matrix metalloproteinase in association with angiogenesis[98].

For initiation of new vessel formation ,lack of oxygen is an important factor.there are two hypoxia inducible substances, which

are HIF-I, and HIF2.Each consists of two subunits ,one is alpha and other is beta subunits.

In premalignant and malignant conditions of breast HIF-1alpha level of expression is high.Tumors with well differentiation the level of HIF-1 alpha is low[98].

Tumor angiogenesis is believed to be co-ordinated by a fine balance between angiogenic activators and inhibitors .Multiple angiogenic factors have been implicated as positive regulators of angiogenesis,such as VEGF and bFGF.

MICROVESSEL DENSITY:

Microvessel density (MVD) has become the morphological gold –standard for assessing the neovacularization in tumors[99]. Several studies have demonstrated that the MVD is closely correlated with the prognosis of breast cancer. It has been demonstrated that increasing density of newly formed micro vessels in growing tumors correlated closely with increasing number of tumor cells shed into the blood stream.

In recent years, mounting evidence has suggested that quantification of microvessel density (MVD) by immunostaining for

endothelial cell markers, such as CD34,CD31,von Willebrand factor (vWF) may be a useful prognostic predictor in cancer patients. CD34 is a transmembrane glycoprotein expressed by haematopoietic stem cells, endothelial cells and mesenchymal cells in different tissues including breast.

Fibrocystic breast disease with the highest vascular density are associated with a greater risk of breast carcinoma[101]. High microvessel density has been associated with a poor grade of histologic differentiation in invasive duct carcinomas and high risk of axillary nodal metastasis[72] and low survival rate.

MEASUREMENT OF MICROVESSEL DENSITY :

As early in 1972, Brem et al. proposed a microscopic angiogenesis grading system to assess the angiogenic status of the tumor vasculature .In 1991, Weidner et al[71] described a new method to assess microvascular density.

The evaluation of staining with CD34 (microvessel density; MVD) was performed by capillary counting in the 3 most highly vascularized areas initially selected (hot spots) under 40x field. Then a 400x field was used to count microvessels in each of these areas.

Single or clusters of endothelial cells, with or without lumen, were considered to be individual vessels. Large vessel with muscular wall, microvessel in necrotic area and periphery of the tumor field were excluded.

In a study made by H P Dhakal et a and Fox B et al the Chalkley method appears to be the improved method in estimating the prognostic impact of vascularity in invasive carcinoma of breast.

Microscopic analysis for areas of micro vessel reflects intra tumoral vascularization and hence its malignant potential.

A study by Weidner et al[71] shows that angiogenesis of breast carcinoma has prognostic significance.

In these studies, angiogenesis was compared to a number of clinicopathological parameters such as patient age, tumour size, histological grade, lymph node status, estrogen receptor status and prognosis.

MEASURE MENT OF MICROVESSEL DENSITY:

Three different methods used to assess MVD

METHOD 1 :

1. Visual scanning of stained slides at all the magnification (100x,400x)
2. Semiquantitative grading for CD34 staining.
3. Review of the slides by 2 experienced pathologists to determine the accuracy of this method
4. To ensure vessel specific staining prior histological has to be done.

METHOD 2:

1. Scanning of slide at 100x magnification
2. Select three areas with lot of blood vessels(Hot spot)
3. Absolute number of blood vessels is determined at 400x .
4. Average of 3 separate visual counts ,lack of larger vessel and sinusoid was taken as microvessel density.

METHOD 3:

In this method, microvessel density was estimated by using computerized image analysis. The three hot spots used for the visual count is quantified by computer-based image analysis. The PC

compatible image analysis software can be used for analysis of digitally captured images .By using computerized pixel counting , microvessel surface area is calculated and expressed as the percentage of hot spot occupied by CD 34 immuno staining.

According to Indian journal of pathology and microbiology, Maha Mohammed Amin,Zeinab et al[87] the scoring of microvessel density is done as follows

MICRO VESSEL DENSITY SCORING:(MVD GRADE)

Mild MVD - 4-10 capillary

Moderate MVD- 11-20 capillary

Severe MVD - 21-28 capillary

ELASTOSIS:

The presence of elastosis or elastic tissue in the stroma of sections from breast carcinoma was first described by Cheatle and Cutler.

Elastosis is the presence of focal deposits of elastic tissue in abnormal amounts .The significance and pathogenesis of elastosis in breast carcinoma is unknown[88]. It is common in infiltrating ductal and lobular carcinomas of the breast. Elastic tissue is probably produced by

fibroblasts, smooth muscle cells or myofibroblasts, under the simulation of the infiltrating cancer cells.

Elastosis was located around carcinomatous tissue mammary neoplastic ducts ,around veins and as scaterred foci in the stroma. Yellow streaks and flecks may be apparent grossly and probably reflect the increased elastic tissue. In H&E stained sections, elastosis is seen as a homogenous material with a faintly visible fibrillar appearance[88].

In sections stained with the Verhoeff-van gieson method, showed densely packed black colored elastin fibrils. Periductal and stromal elastosis are usually observed in invasive carcinoma of breast.[88]

Epithelial hyperplasia is a premalignant component of fibrocystic breast disease and shows higher degree of elastosis[96] compared to other morphologic changes of fibrocystic disease.The prognostic significance and pathogenesis of elastosis in breast carcinoma is unknown. It is common in infiltrating ductal and lobular carcinomas of the breast. But in recent study breast carcinomas with no associated elastosis have a lower rate of response to endocrine therapy than those with gross elastosis.[97]

In a study by Curt LundMark et al [88] the presence of Elastosis was higher in non-irradiated patients as well asthese who belonged to the age group of 60-69 years. The preoperative management could be probably be a reason for reduction in tissue resulting in condensation of areas with elastosis.

Jackson and Orr et al[100] states,that the condensed elastic tissue could be seen grossly as flecks and streaks of yellow material in all scirrhous breast cancer.In many breast cancer ,the elastosis is relatively difficult to classify histologically with logical accuracy whether the elastosis was a periductal elastin hyperplasia or a new development of elastin fibrils not related with the ductal walls.

ELASTOSIS GRADE:

According to the study by Curt Lundmark[88] grade of elastosis followed

Grade 0 - No elastosis

Grade 1- Mild to moderate degree of elastosis

Grade 3- Gross elastosis

MATERIALS AND METHODS

STUDY DESIGN :

Descriptive study

PLACE OF STUDY :

Department of pathology ,Coimbatore medical college hospital Coimbatore.

STUDY PERIOD :

April 2013 –July 2014

INCLUSION CRITERIA :

All breast specimens including mastectomy and breast biopsy specimen received in the deparment of pathology,Coimbatore medical college hospital.

EXCLUSION CRITERIA:

1) Patient on chemotherapy and radiotherapy
2) Male breast specimens
3) Ill fixed specimens

All mastectomy specimens will be received in 10% formalin. These were grossed and macroscopic features noted. The tissue was then processed routinely paraffin sections were cut at 4 to 5 μm

thickness and stained by H& E ,Verhoeff van Gieson stain and then immunohistochemical study for CD34 was done.

HEMATOXYLIN AND EOSIN STAINING PROCEDURE

REAGENTS :

1) Erhlich,s Hematoxylin solution
2) Eosin Y solution 1%
3) Acid alcohol solution 1%

TECHNIQUE :

1. Deparaffinise the sections
2. The sections are immersed in xylene for thirty seconds
3. Place in isoprophyl alcohol for fifteen minutes
4. Tap water wash for 5 minutes
5. Sections are stained with Erhlich,s Hematoxylin for ten to fifteen minutes and washed in top water
6. Differentiate in 1% acid alcohol solution – 2 to 3 dips
7. Then blueing for ten minutes
8. Counterstain wih 1% Eosin solution -2 to 3 dips
9. Tap water wash for 5 minutes
10. Sections are air dried
11. Finally dipped in xylene
12. DPX mounting

VERHOEFF-VAN GIESON STAINING TECHNIQUE:

REAGENTS:

SOLUTION-A

1) Haemotoxylin- 5 gm
2) Absolute alcohol- 100ml

SOLUTION-B

1) Ferric chloride- 10gm
2) Distilled water- 100ml

SOLUTION-C- (LUGOLS IODINE SOLUTION)

1) Iodine - 1gm
2) Potassium iodide - 2gm
3) Distilled water - 100ml

WORKING SOLUTION-D

Solution (A) - 2oml

Solution (B) - 8ml

Solution (C) - 8ml

Add in the above order and between addition mix.

PROCEDURE:

1) Dewax sections and bring to water
2) Cover with staining solution for 30 minutes
3) Rinse in water

4) Differentiate in 2% aqueus ferric chloride until elastic fibres apper black on grey background.
5) Rinse in water
6) Dip in 95% alcohol to remove any staining due to iodine alone
7) Counterstain with Van Gieson solution for 1-2 minutes
8) Blot dry to remove excess stain (don't wash)
9) Dehydrate clean and mount.

RESULTS:

Elastic tissue fibers – black

Other tissue elements - yellow

METHODS OF IMMUNOHISTOCHEMISTRY:

This method involves in two step indirect technique.

1) The primary antibody binds with specific epitopes.
2) This is followed by detection of antigen –antibody reaction – called caloriemetric reaction.

REAGENTS USED:

1. Peroxide block :3% hydrogen peroxide in water
2. Power block reagent
3. Chromogen:DAB substrate-3,3-diaminobenzidine
4. Liquid DAB substrate
5. Reagent of super enhancer

6. Poly-HPR Reagent
7. Mayers hematoxylin – counter stain
8. Buffer solutions

BUFFERS USED IN IHC

1.TRIS EDTA: pH-9.0

TRIS buffer salt: 6.05 gm

Disodium EDTA: 0.744 gm

Distilled water: 1000 ml

2. TRIS BUFFER:Ph-6

TRIS buffer salt : 0.605 gm

Sodium chloride: 8 gm

Distilled water: 1000 ml

1N hydrochloric acid- 3ml

3. CITRATE BUFFER: pH-6

Trisodium citrate : 2.94 gm

Distilled water: 1000 ml

1N hydrochloric acid: 5 ml

IHC PROCEDURE METHOD:

1) First tissue sections are deparaffinised in xylene for 30 minutes
2) Then absolute alcohol wash for five minutes with two changes
3) Follows tap water wash for ten minutes

4) Rinse in distilled water for five minutes

5) Then retrieval of antigen is done by placing the slides is placed in microwave with appropriate buffer solutions

6) Cool in room temperature then rinse in distilled water

7) Wash in buffer of TBS for 5minutes with two changes

8) Treat with peroxide block for ten minutes

9) Wash in buffer of TBS for five minutes with two changes

10) Treat with peroxide block for ten minutes

11) Then drain the slides treat with primary antibody

12) Wash in buffer of TBS for five minutes with two changes

13) Then cover the slides with superenhancer for thirty minutes

14) Wash in buffer of TBS for five minutes with two changes

15) Then apply reagent of poly HRP and leave for thirty minutes

16) Wash in buffer of TBS for five minutes with two changes

17) Then treat with DAB chromogen with substrate buffer for five to eight minutes.

18) Wash in buffer of TBS for five minutes with two changes

19) Tap water wash for five minutes

20) Couterstain with mayers hematoxylin for one minute

21) Then tap water wash for five minutes

22) Air dry and mount in DPX

OBSERVATIONS AND RESULTS

ANALYSIS OF FIBROCYSTIC BREAST DISEASE CASES

Total of 30 cases were studied and the following observations were obtained.

Table : 1

Distribution of Fibrocystic breast disease cases according to different age groups

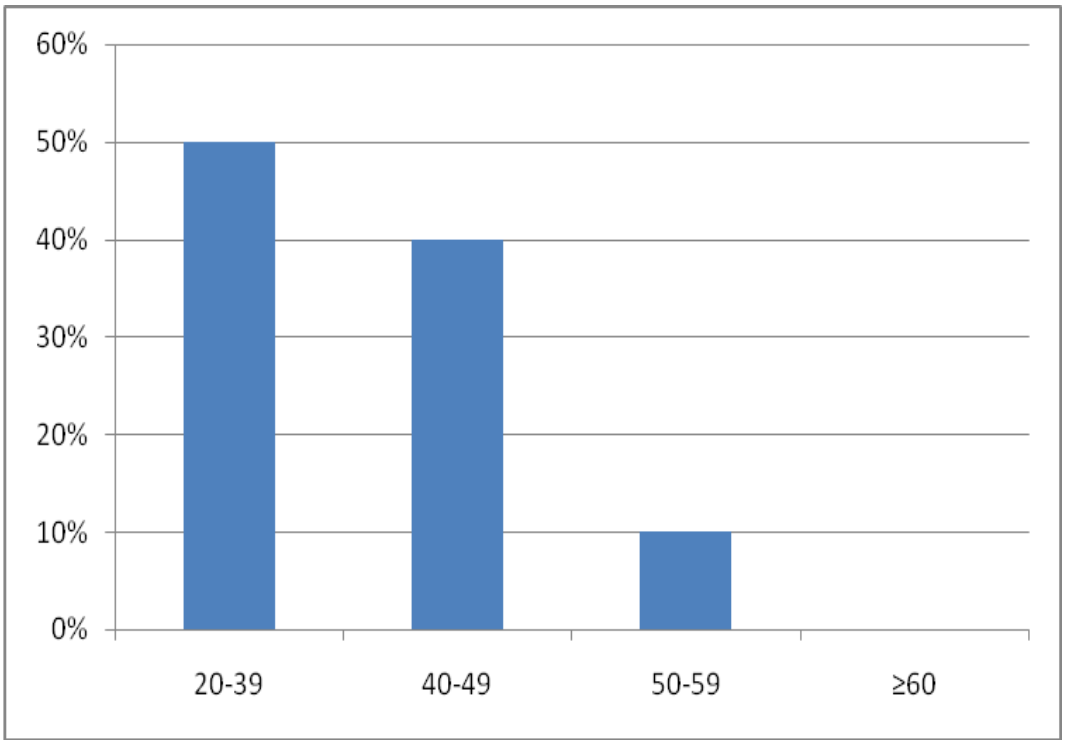
AGE(Years)	NUMBER	PERCENTAGE (%)
20-39	15	50%
40-49	12	40%
50-59	3	10%
≥60	0	0%

Majority of Fibrocystic breast disease cases belonged to 20-39 years of age group (50%)

Chart : 1

Distribution of Fibrocystic breast disease cases according to different age groups

.



The peak age group of fibrocystic breast disease is 20-39 years.

Table : 2

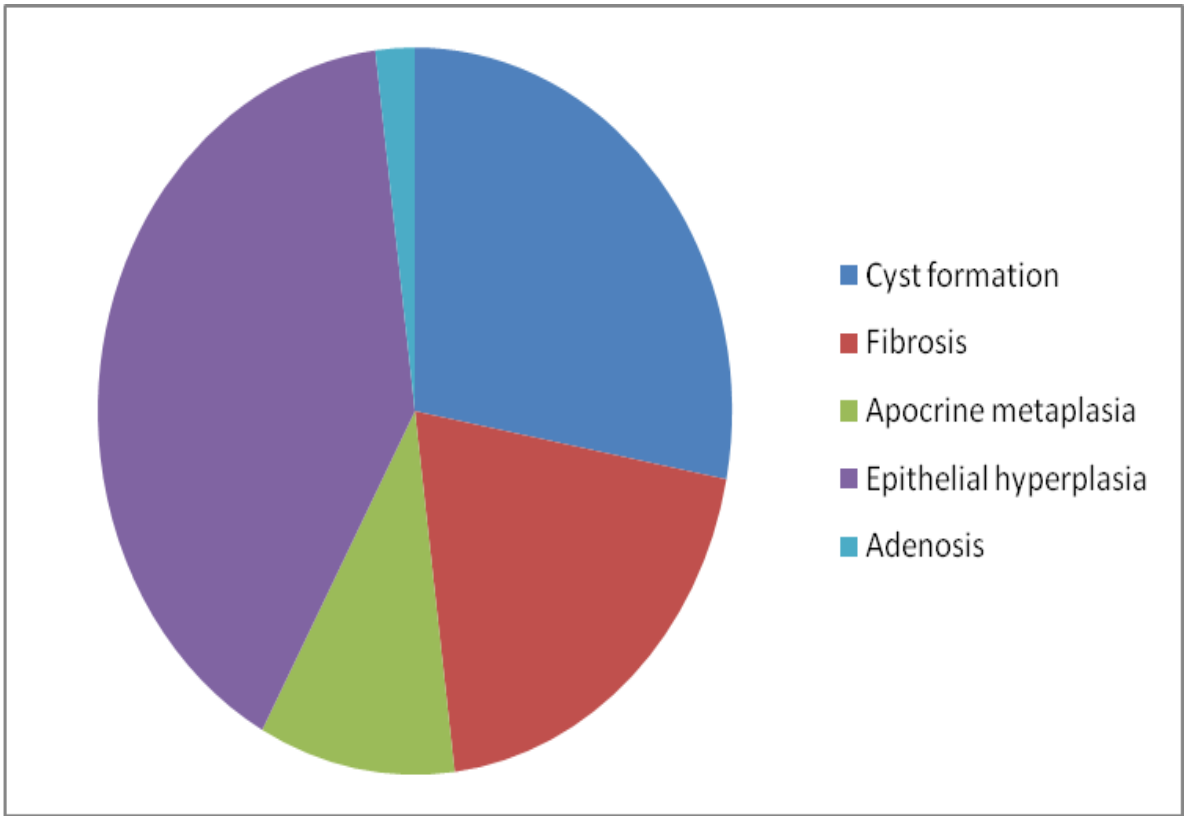
Distribution of morphological changes in Fibrocystic breast disease

MORPHOLOGICAL CHANGES	NUMBER
Cyst formation	14
Fibrosis	10
Apocrine metaplasia	5
Ductal epithelial hyperplasia	20
Adenosis	1
Calcification	Not seen
Chronic inflammation	Not seen
Fibroadenomatoid change	Not seen

The most common morphological changes were cyst formation, epithelial hyperplasia and fibrosis.

Chart : 2

Distribution of morphological changes in Fibrocystic breast disease



The most common morphological change were ductal epithelial hyperplasia,cyst formation and fibrosis.

Table : 3

Correlation of epithelial hyperplasia and grade of elastosis

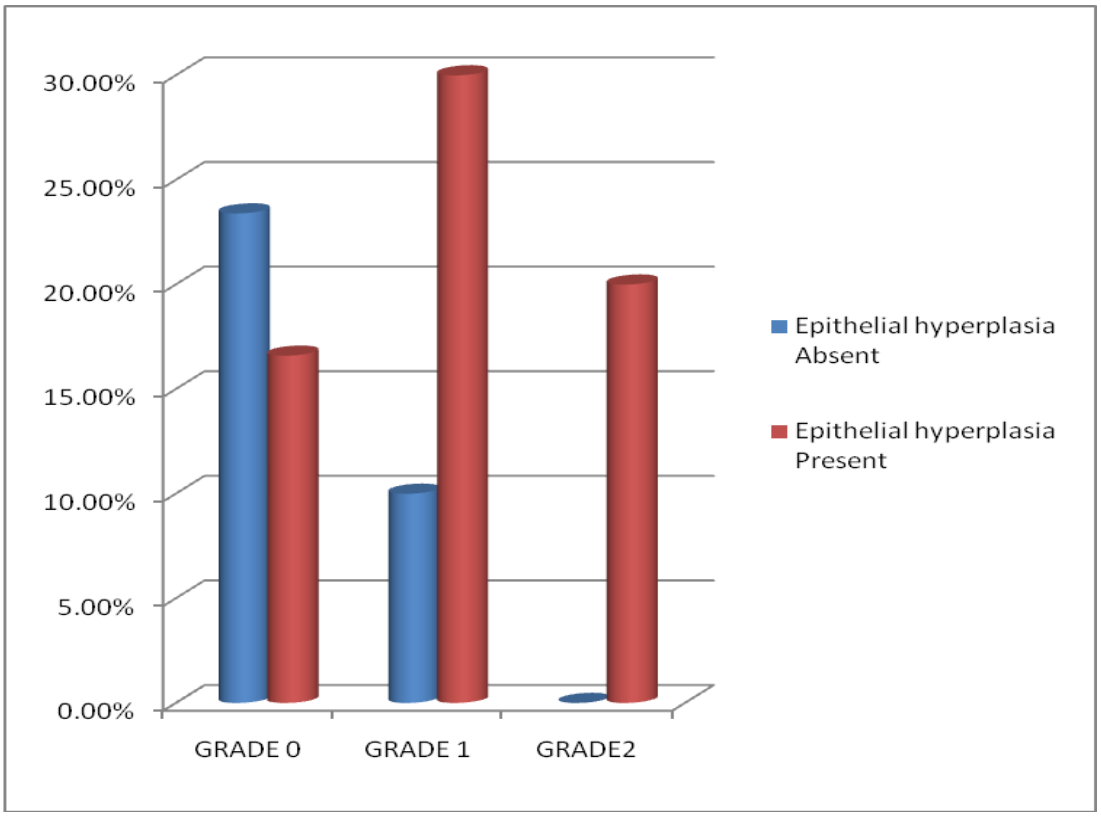
MORPHOLOGY	ELASTOSIS GRADE			
	GRADE 0	**GRADE 1**	**GRADE 2**	**Total**
Epithelial hyperplasia Absent	7(23.4%)	3(10%)	0(0%)	10(33.4%)
Epithelial hyperplasia Present	5(16.6%)	9(30%)	6(20%)	20(66.6%)
Total	**12(40%)**	**12(40%)**	**6(20%)**	**30(100%)**

Chi-square total=0.230 (p value>0.05)

In this study Elastosis is more commonly associated with epithelial hyperplasia.

Chart : 3

Correlation of epithelial hyperplasia and grade of elastosis



ELASTOSIS GRADE

In this study the correlation between epithelial hyperplasia and grade of elastosis was statistically not significant (pvalue>0.05)

Table : 4

Correlation of epithelial hyperplasia with micro vessel density score (grade)

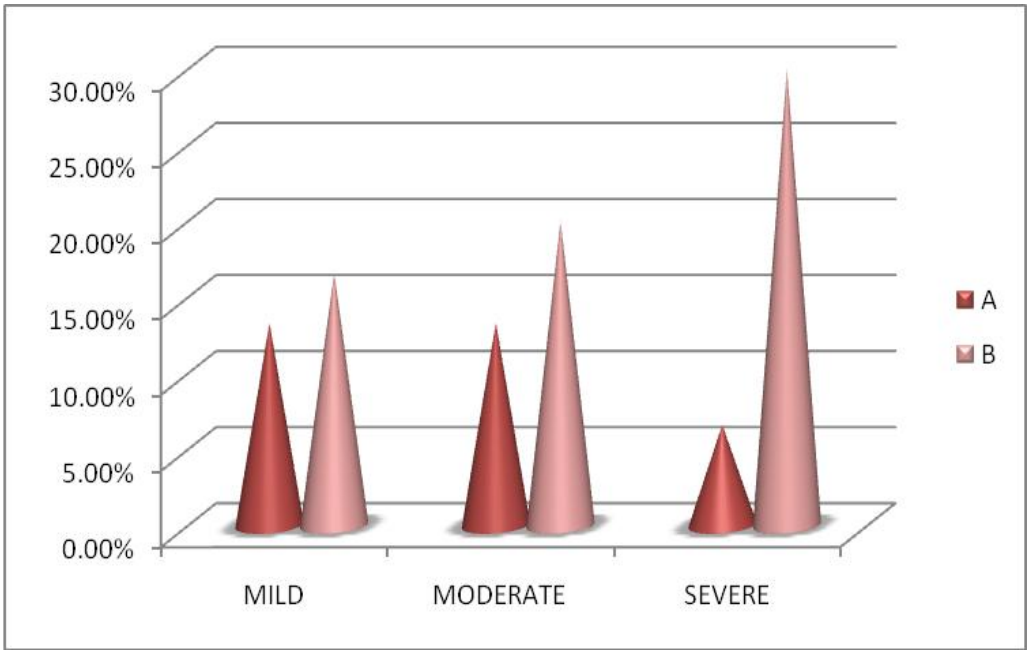
MORPHOLOGY	MVD SCORE (Grade)			
	MILD	**MODERATE**	**SEVERE**	**Total**
Epithelial hyperplasia Absent	4(13.3%)	4(13.3%)	2(6.6%)	10(33.3%)
Epithelial hyperplasia Present	5(16.6%)	6(20%)	9(30%)	20(66.7%)
Total	**9(30%)**	**10(33.4%)**	**11(36.6%)**	**30(100%)**

Chi-square total=0.001 ($p<0.05$)

In this study that severe grade of Microvessel density associated with epithelial hyperplasia.This is statistically significant.

Chart : 4

Correlation of epithelial hyperplasia with micro vessel density score (grade)



MICROVESSEL DENSITY SCORE(GRADE)

A= EPITHELIAL HYPERPLASIA ABSENT

B= EPIYHELIAL HYPERPLASIA PRESENT

ANALYSIS OF INVASIVE DUCTAL CARCINOMA-NOS TYPE CASES

Total of 30 cases were studied and following results obtained

Table: 5

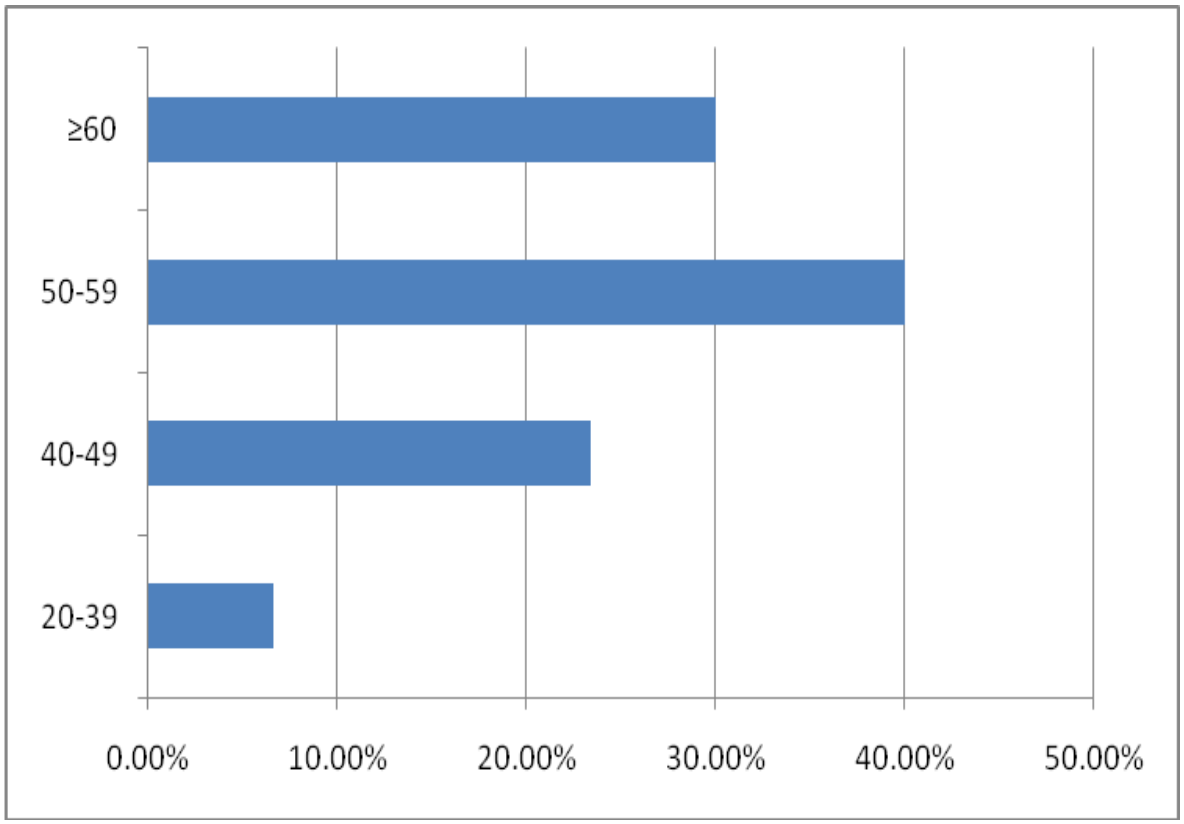
Distribution of Invasive ductal carcinoma-Nos type cases according to different age groups

AGE (Years)	NUMBER	PERCENTAGE (%)
20-39	2	6.6%
40-49	7	23.4%
50-59	12	40%
≥60	9	30%

In the table majority of Invasive ducctal carcinoma-Nos type cases belonged to 50-59 years of age groups (40%)

Chart : 5

Distribution of invasive ductal carcinoma-nos type cases according to different age groups



(IDC-NOS)

The peak age group of Invasive ductal carcinoma –Nos type cases -50-59 years.

Table : 6

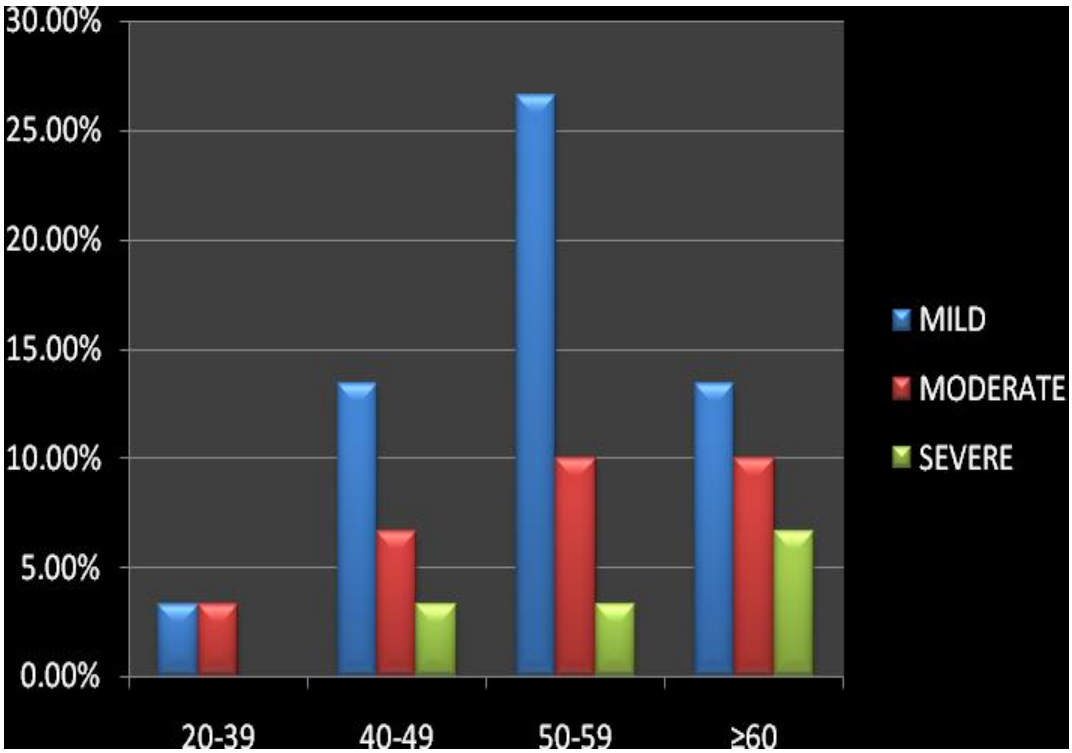
Correlation of Micro vessel density grade with the age of the Invasive duct carcinoma-Nos type cases

AGE (Years)	MVD SCORE (GRADE)			
	MILD	**MODERATE**	**SEVERE**	**Total**
20-39	1(3.3%)	1(3.3%)	0(0%)	2(6.6%)
40-49	4(13.4%)	2(6.6%)	1(3.3%)	7(23.4%)
50-59	8(26.6%)	3(10%)	1(3.3%)	12(40%)
≥60	4(13.4%)	3(10%)	2(6.6%)	9(30%)
TOTAL	**17(56.6%)**	**10(33.4%)**	**3(10%)**	**30(100%)**

No significant correlation between Microvessel density grade and age of Invasive ductal carcinoma cases was noted.

Chart : 6

Correlation of Micro vessel density grade with the age of the Invasive duct carcinoma-Nos type cases



This table shoes that the grade of microvessel density does not correlate with age of occurrence of invasive ductal carcinoma-nos type.

Table : 7

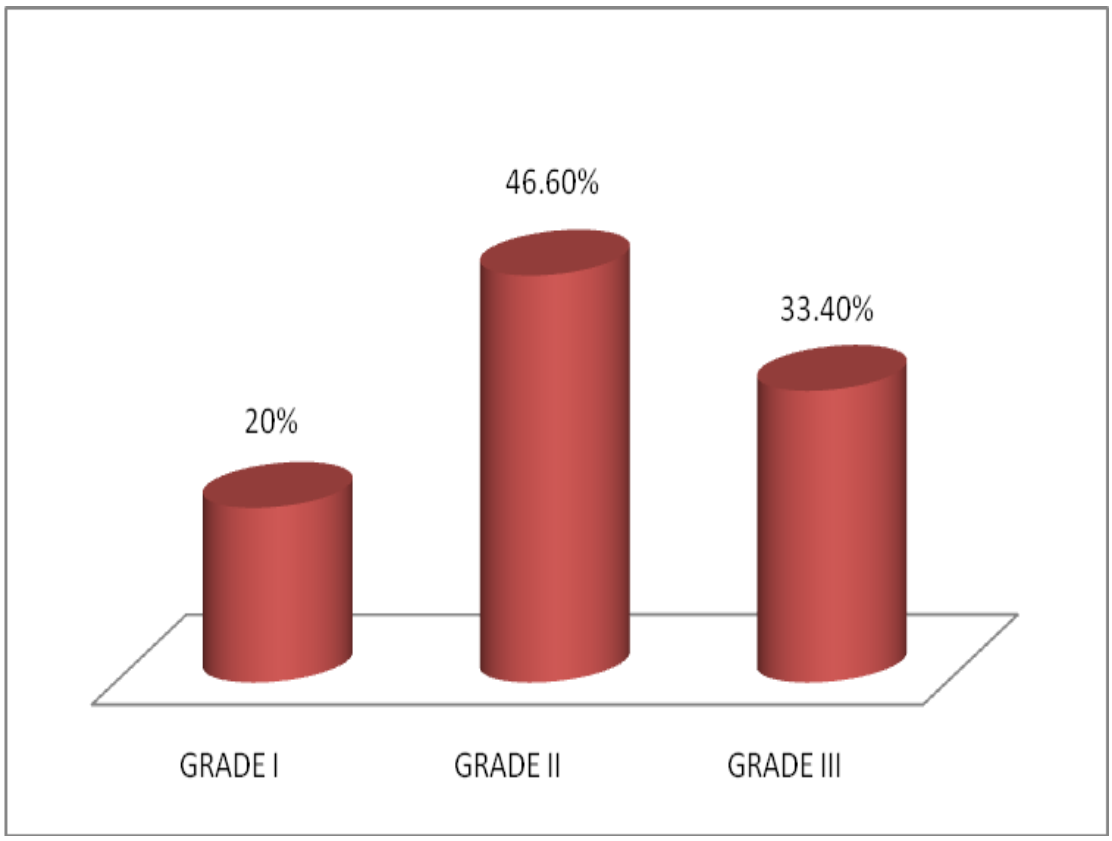
Distribution of Invasive ductal carcinoma-Nos type cases according to histological grade

HISTOLOGICAL GRADE	NUMBER	PERCENTAGE (%)
GRADE I	6	20%
GRADE II	14	46.6%
GRADE III	10	33.4%

Majority of Invasive ductal carcinoma cases were histological gradeII.

Chart : 7

Distribution of Invasive ductal carcinoma-Nos type cases according to histological grade



HISTOLOGIC GRADE

Majority of tumors were histologically grade II(46.6)

Table : 8

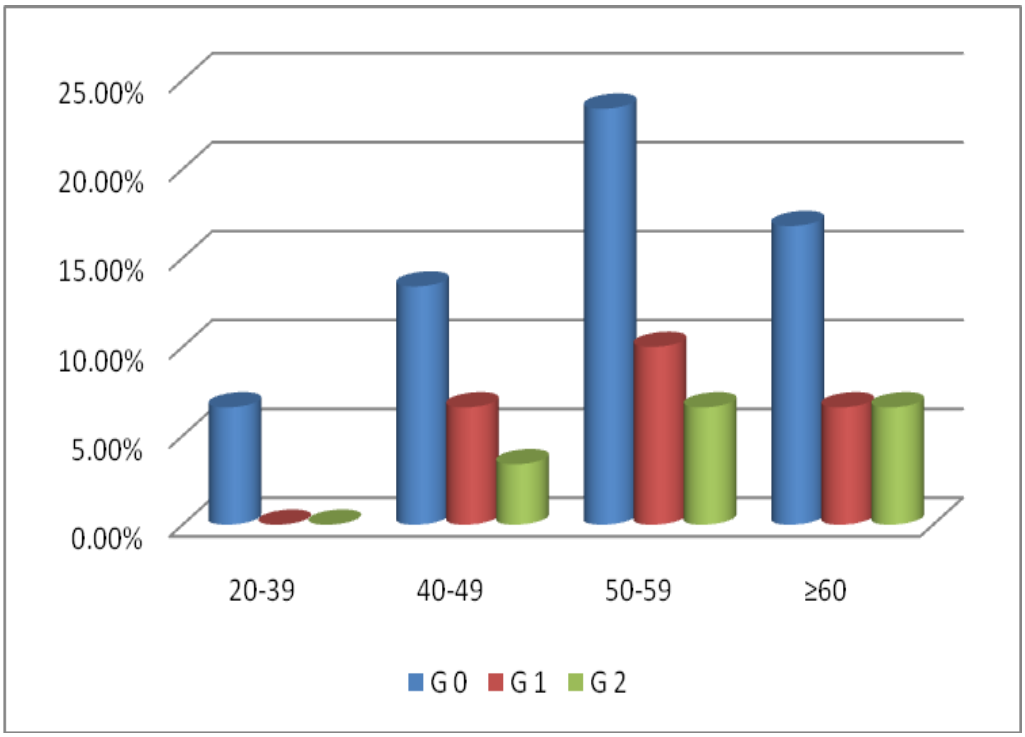
Distribution of Invasive ductal carcinoma-nos type cases with regard to elastosis grade

AGE(Years)	ELASTOSIS (grade)			
	0	**1**	**2**	Total
20-39	2(6.6%)	0(0%)	0(0%)	2(6.6%)
40-49	4(13.4%)	2(6.6%)	1(3.4%)	7(23.4%)
50-59	7(23.4%)	3(10%)	2(6.6%)	12(40%)
≥60	5(16.8%)	2(6.6%)	2(6.6%)	9(30%)
Total	18(60%)	7(23.4%)	5(16.6)	30(100%)

There was no significant association elastosis and age of Invasive ductal carcinoma-Nos type cases.

Chart : 8

Distribution of Invasive ductal carcinoma-nos type cases with regard to elastosis grade



G 0- GRADE 0 ELASTOSIS

G 1- GRADE 1ELASTOSIS

G 2 - GRADE 2 ELASTOSIS

Table : 9

Correlation of histological grade with microvessel density grade

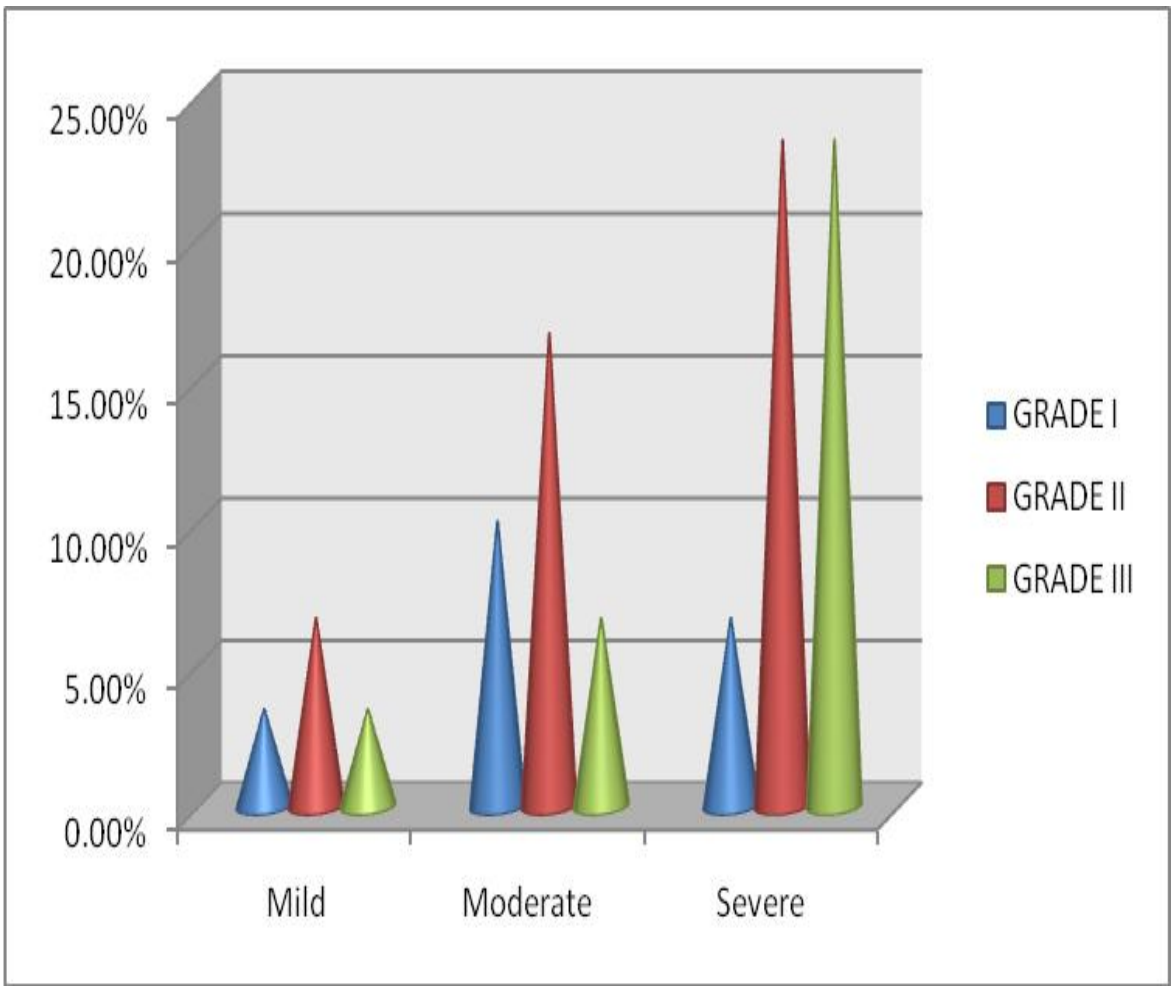
HISTOLOGICAL GRADE	MVD GRADE (SCORE)			
	Mild	**Moderate**	**Severe**	**Total**
GRADE I	1(3.4%)	3(10%)	2(6.6%)	6(4%)
GRADE II	2(6.6%)	5(16.6%)	7(23.4%)	14(56%)
GRADE III	1(3.4%)	2(6.6%)	7(23.4%)	10(40%)
Total	6(20%)	10(33.4%)	14(46.6%)	30(100%)

Chi-square test=0.002 ($p<0.05$)

Severe grade Microvessel density was seen in with grade III tumors and grade II tumors.

Chart : 9

Correlation of histological grade with microvessel density grade



MVD GRADE

The correlation between histological grade with Microvessel density grade is statistically significant.

Table : 10

Correlation of histological grade with elastosis grade

ELASTOSIS GRADE	HISTOLOGICAL GRADE			
	I	**II**	**III**	**Total**
0	3(10%)	10(33.4%)	5(16.6%)	18(60%)
1	2(6.6%)	2(6.6%)	3(10%)	7(23.4%)
2	1(3.4%)	2(6.6%)	2(6.6%)	5(16.6%)
Total	6(20%)	14(46.6%)	10(33.4%)	30(100%)

Chi-square test=0.118($p>0.005$)

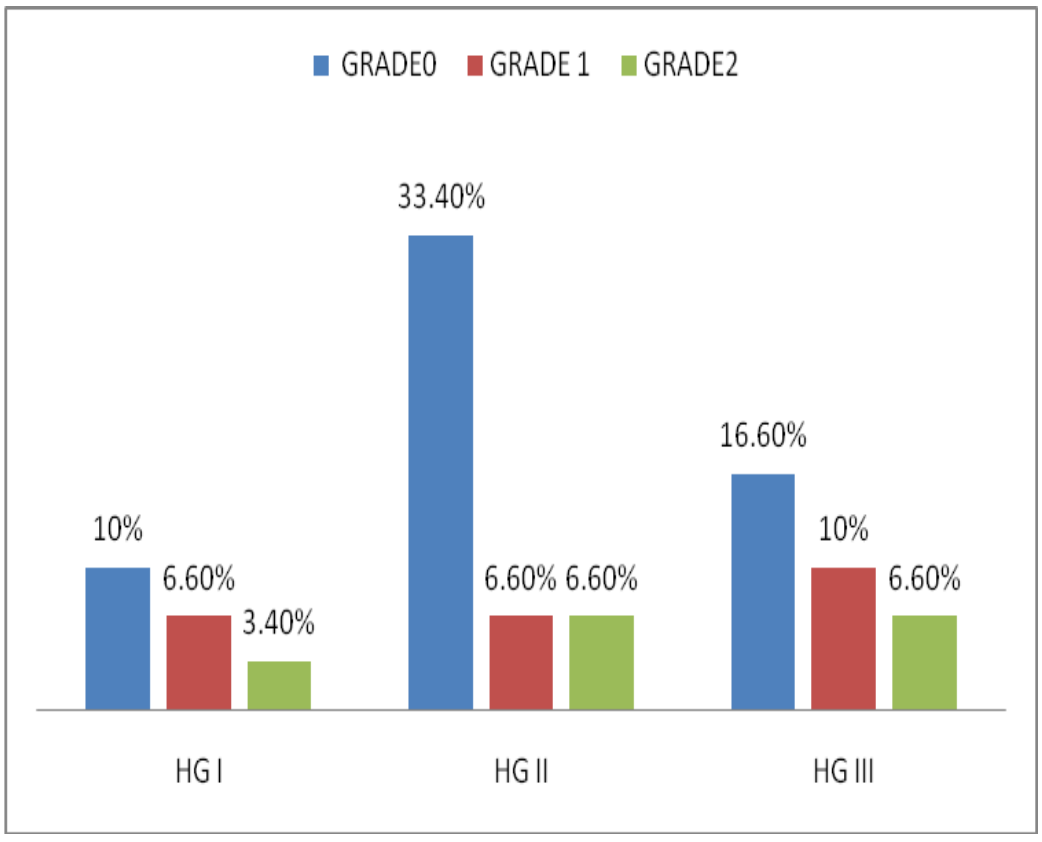
Significant correlation between elastosis and histological grade tumor was not seen.

Chart 10 :

Correlation of histological grade with elastosis grade

HG – HISTOLOGICAL GRADE

GRADE 0/1/2 – ELASTOSIS GRADE



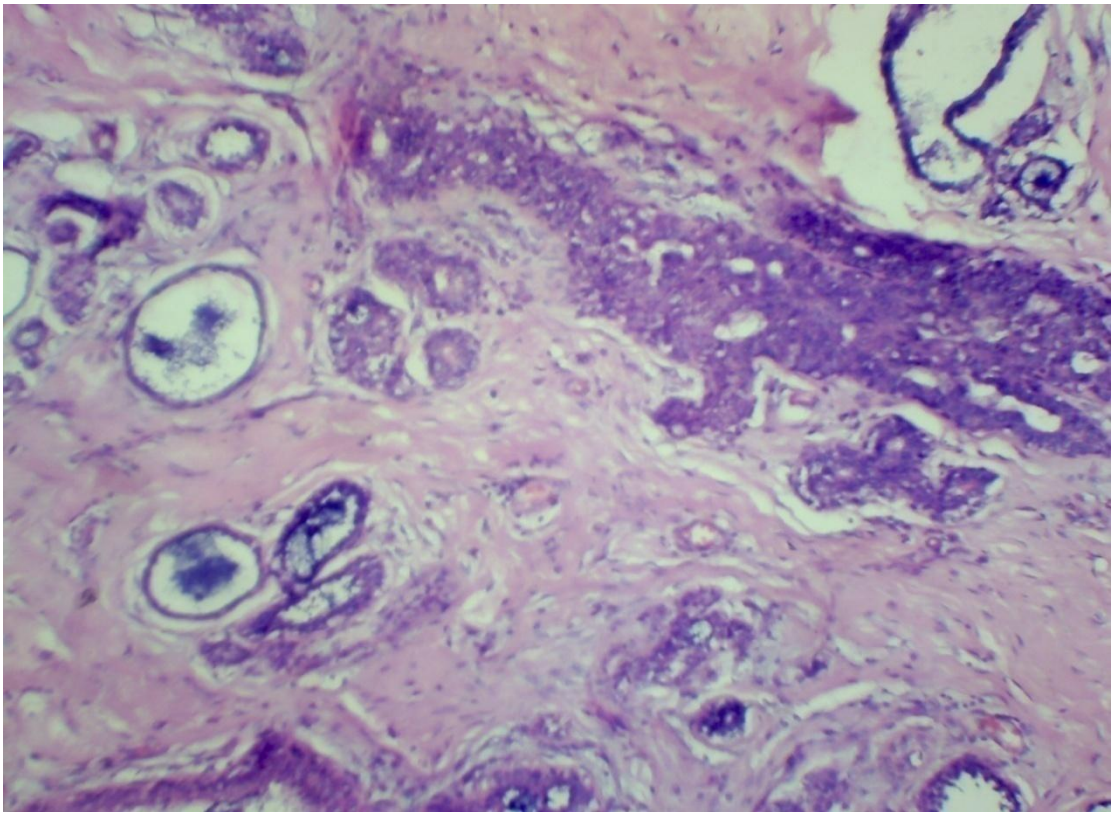


FIG 1 : FCD with Ductal Epithelial Hyperplasia and Cyst formation (H&E Section)

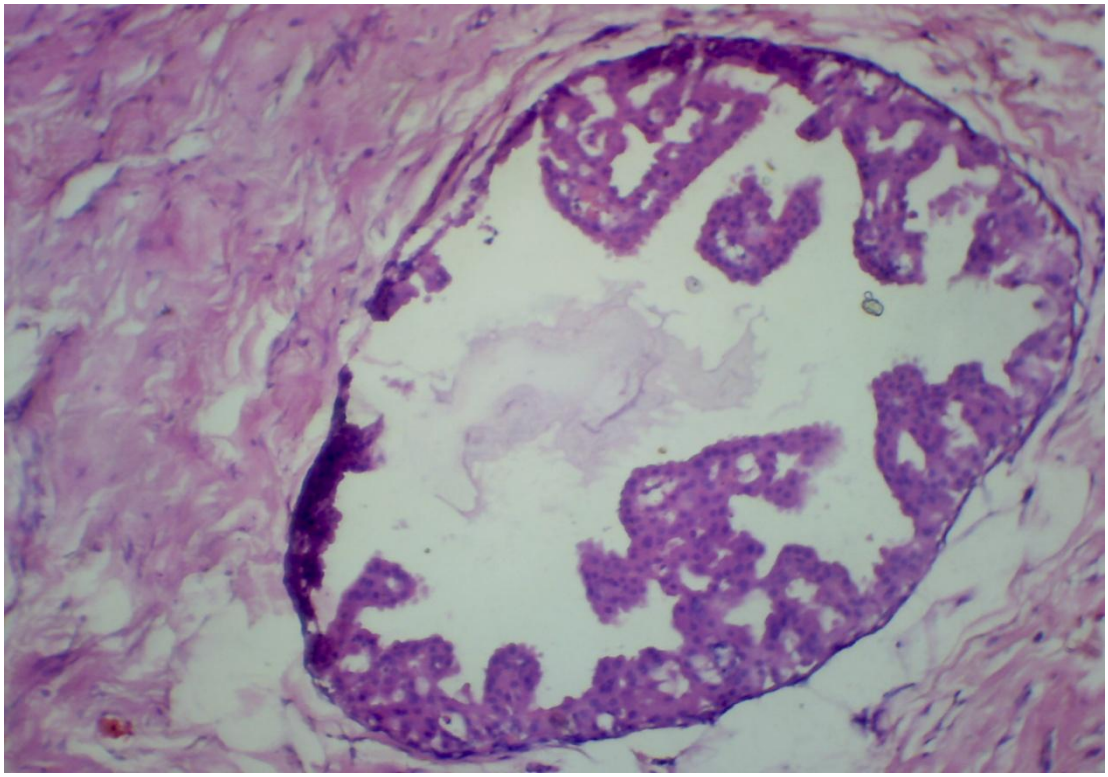


FIG 2 : FCD with Apocrine Metaplasia (H&E Section)

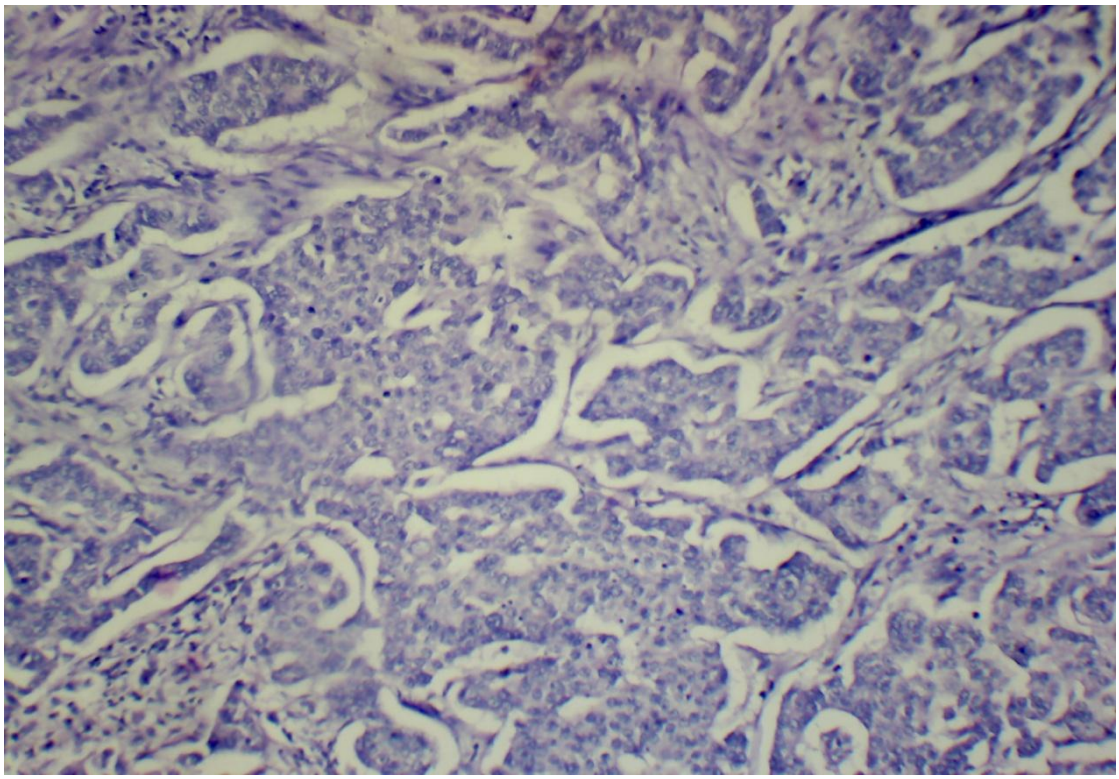


FIG 3 : Invasive Ductal Carcinoma Nos Type (H&E Section)-10 x View

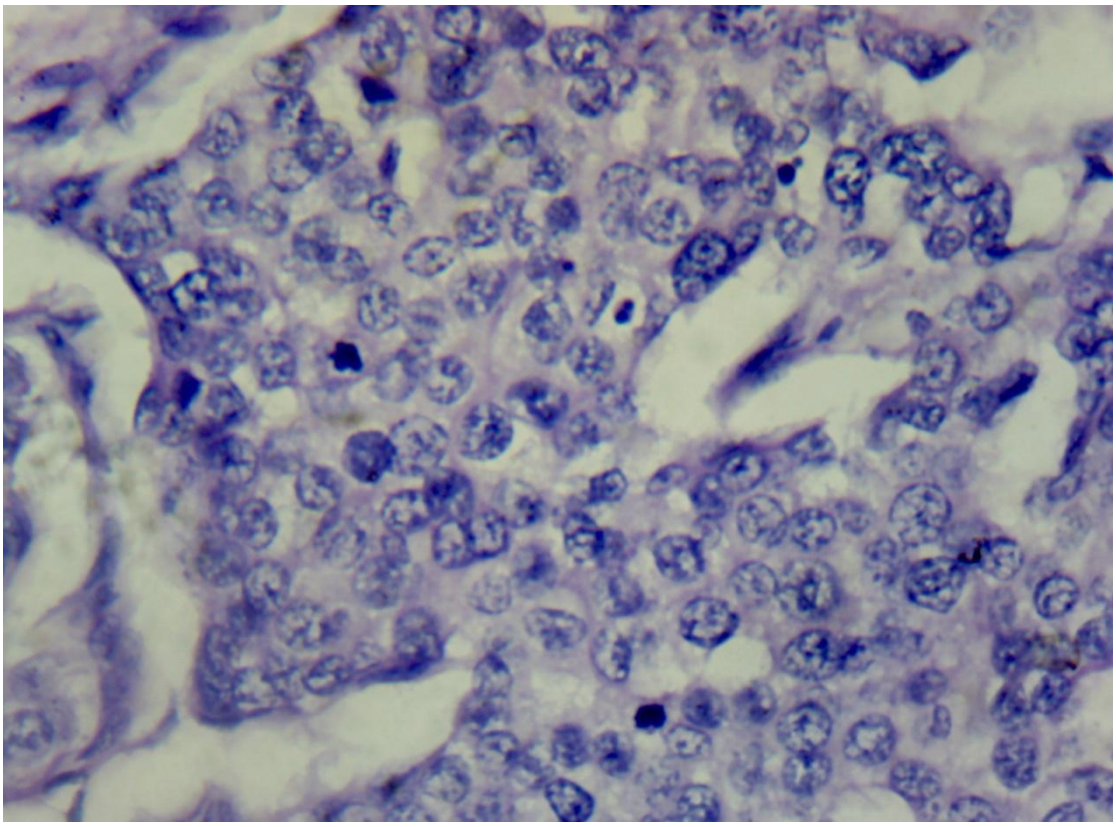


FIG 4 : Invasive Ductal Carcinoma Nos Type (H&E Section) – 40 x view

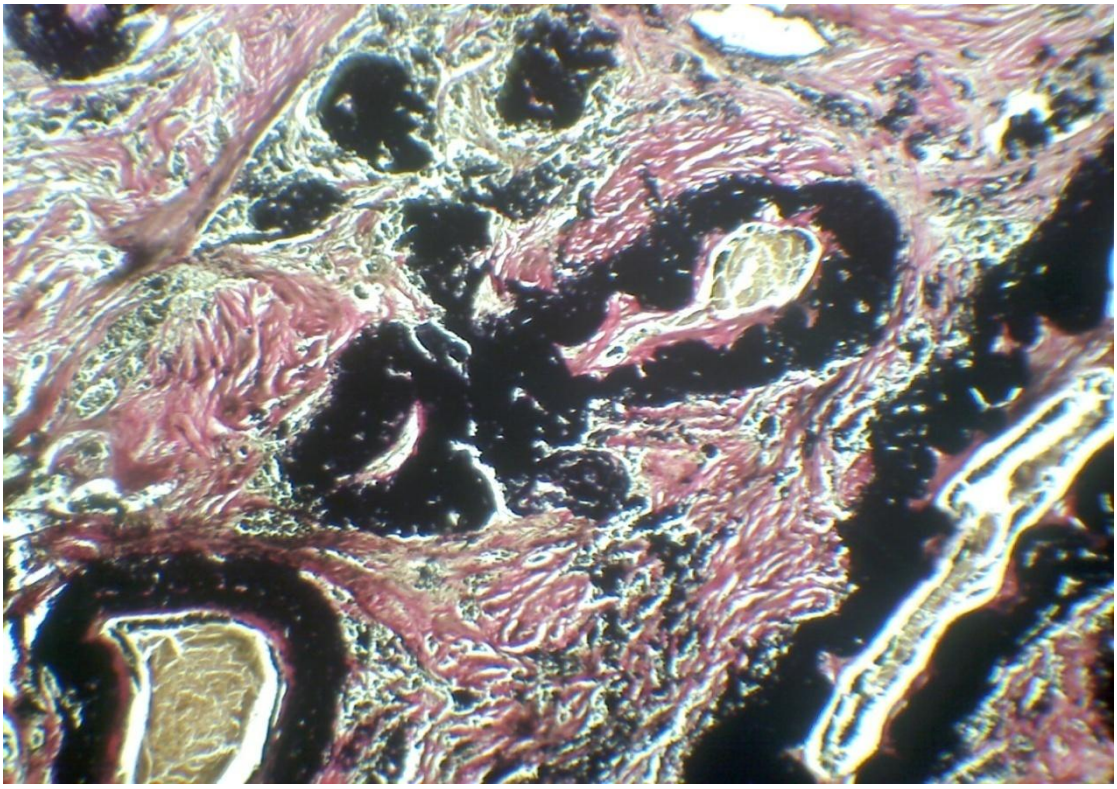


FIG 5: FCD With Periductal Elastosis (VVG Special Stain)

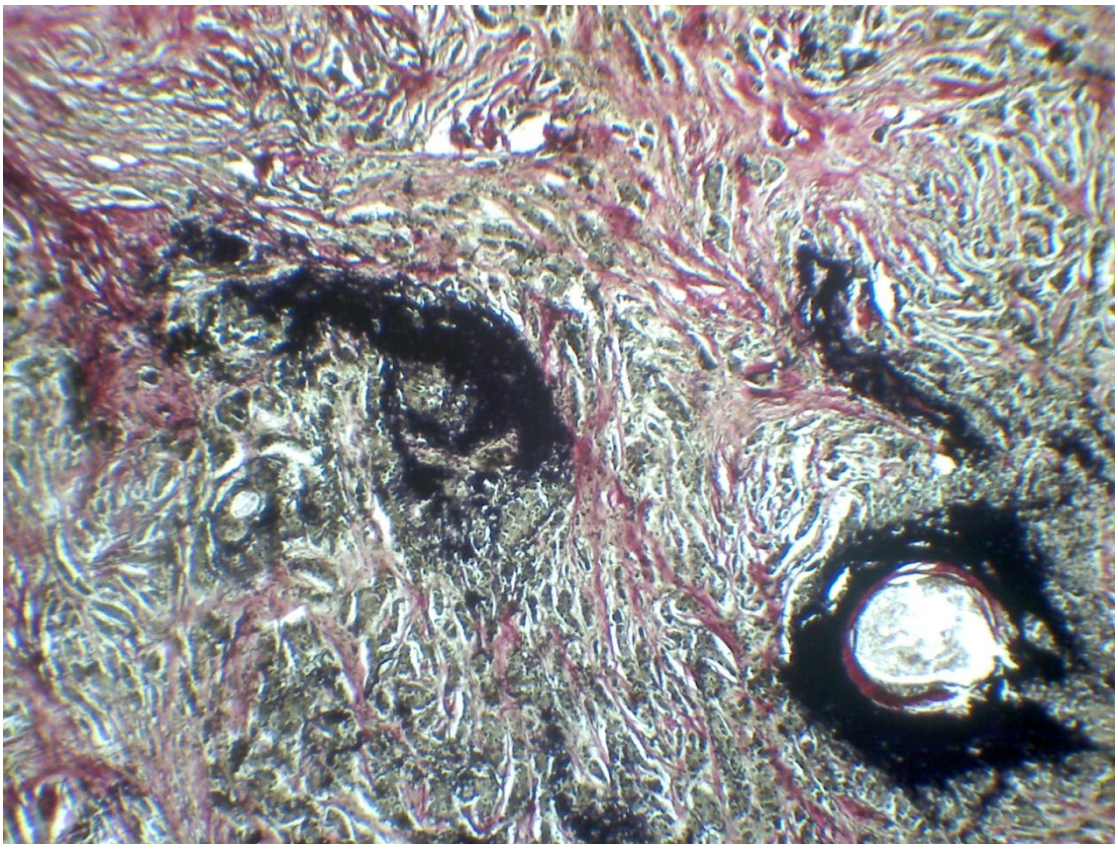


FIG 6 : IDC-NOS with Peridctal and Stromal Elastosis (VVG special stain)

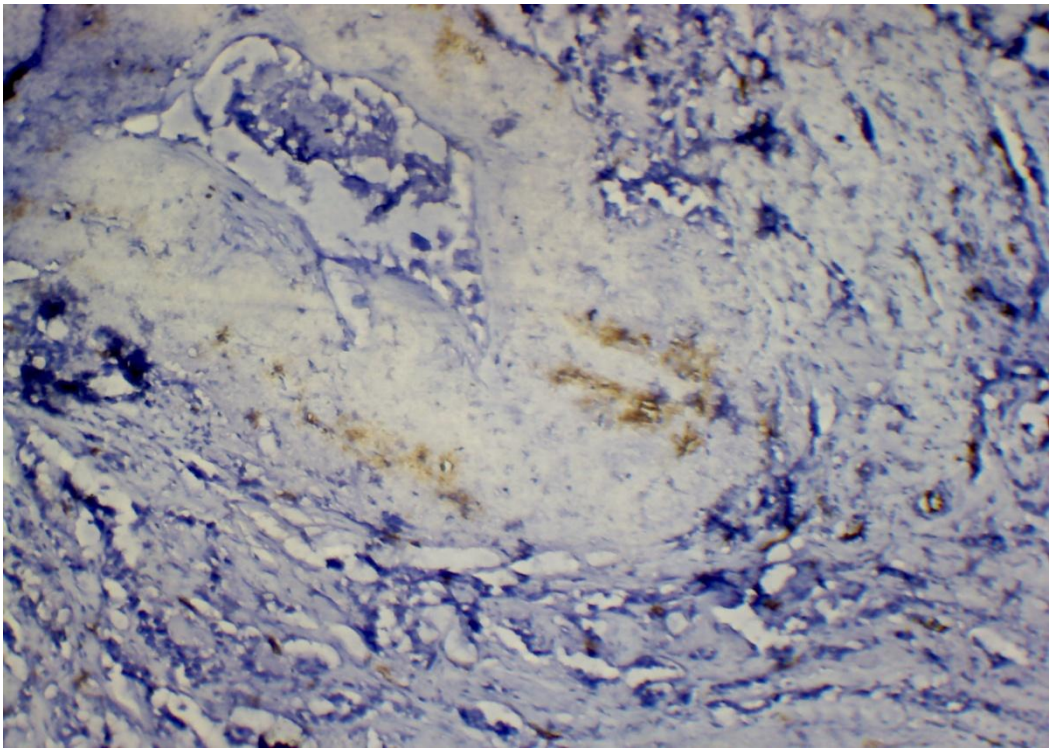


FIG 7: FCD – Micro vessel density CD 34 immuno stain – Mild degree (40X)

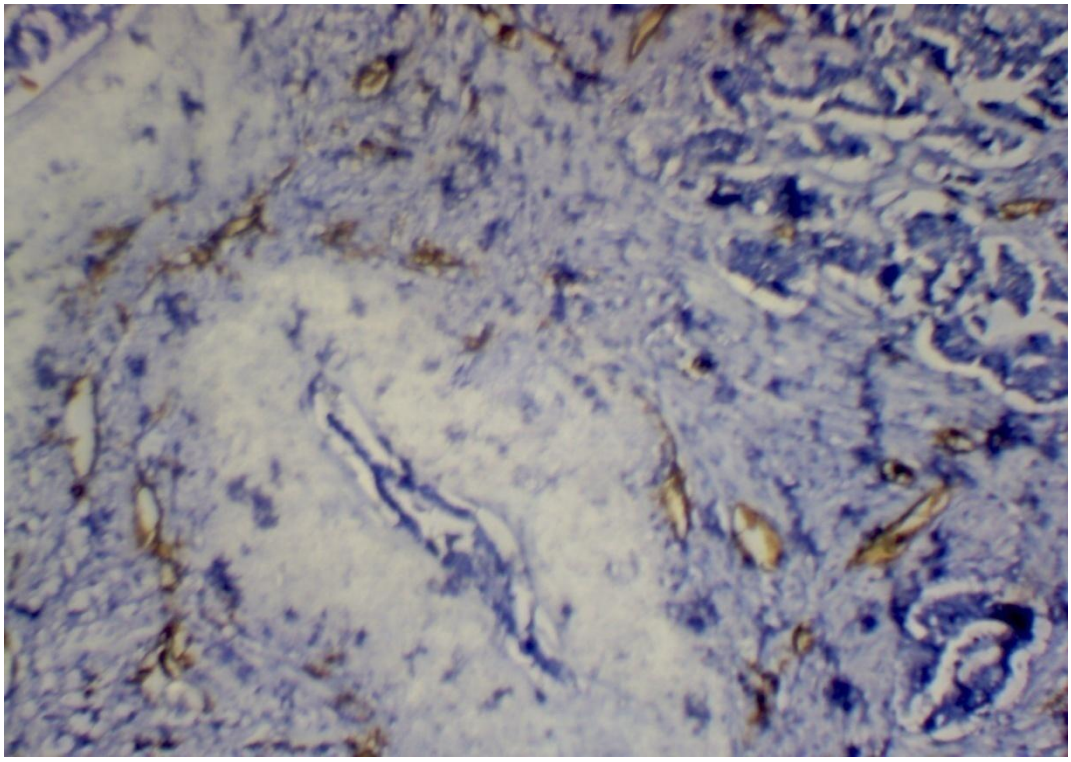


FIG 8 : FCD – Micro vessel density CD 34 immuno stain – Severe degree (40X)

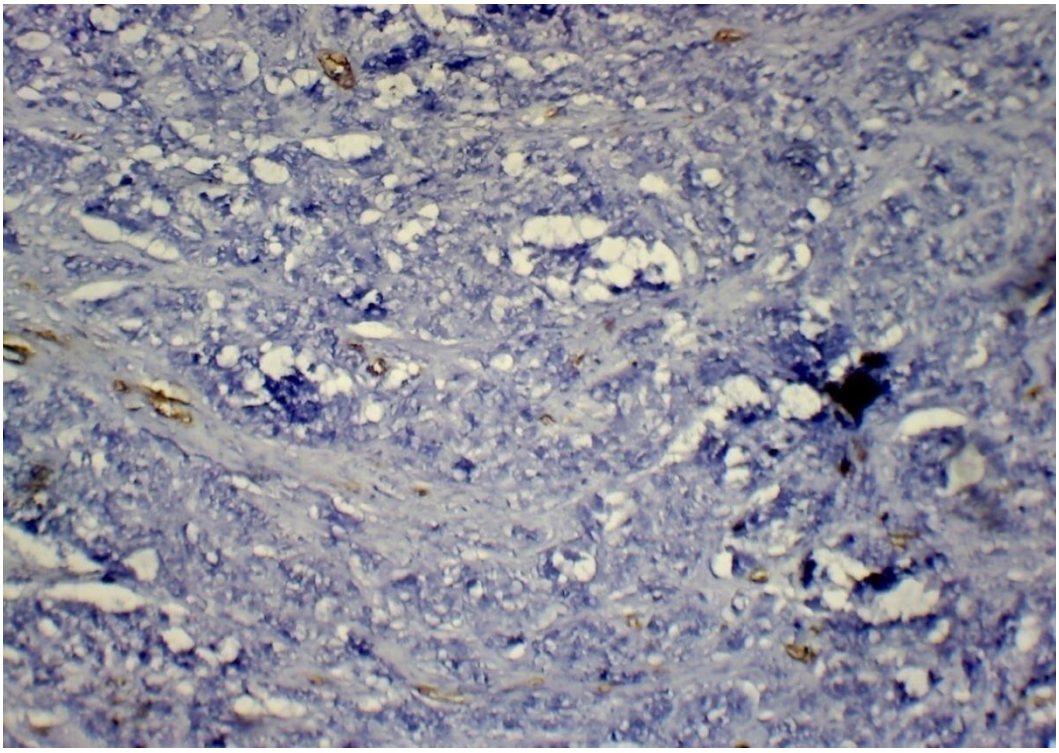


FIG 9: IDC-NOS Micro vessel density CD 34 immuno stain – Mild degree (10X)

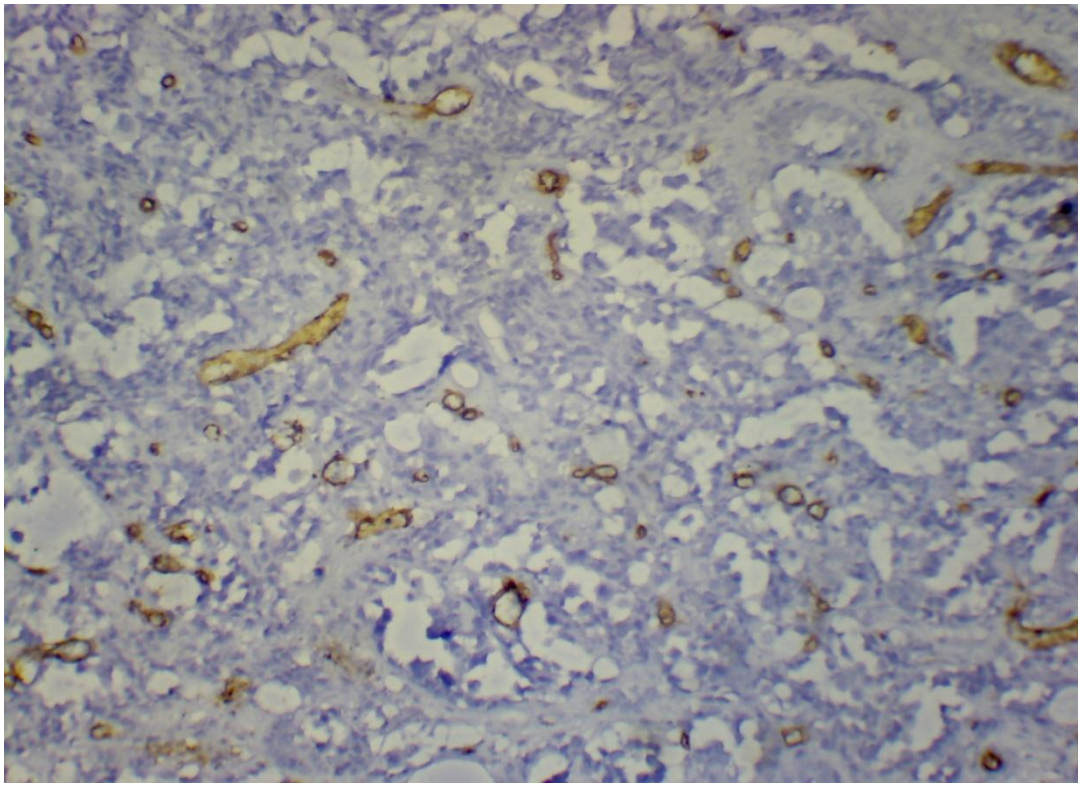


FIG 10: IDC-NOS Micro vessel density CD 34 immuno stain – moderate degree (10X)

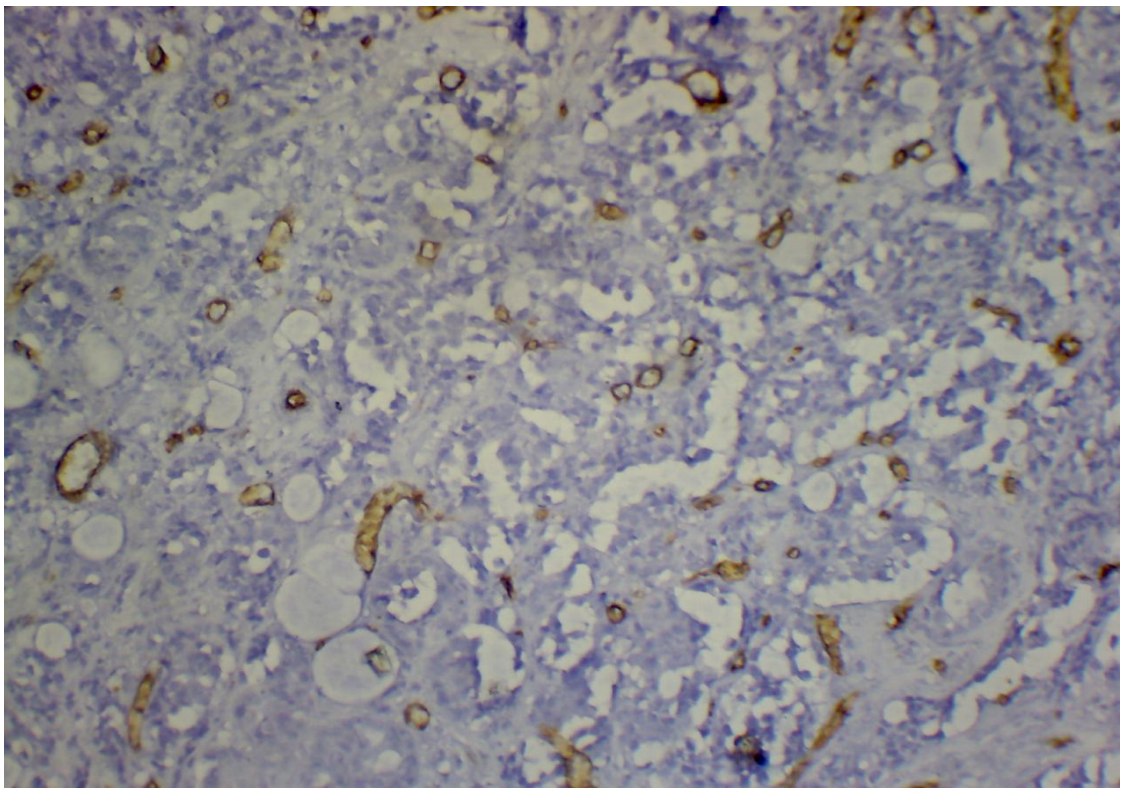


FIG 11 : IDC-NOS Micro vessel density CD 34 immuno stain – Severe degree (10X)

DISCUSSION

Fibrocystic breast disease is a benign condition of the breast due to exaggerated hormonally mediated response.The peak age of occurrence is between 30-50years.

In this present study majority of Fibrocystic breast disease cases belonged to age of 20-39 years (50%).This correlated with a study done by Shaaban AM, Sloane JP[102] et al.

In the present study the most common morphological changes of Fibrocystic breast disease were cyst formation and epithelial hyperplasia followed by fibrosis, apocrine metaplasia, and adenosis. Ductal epithelial hyperplasia poses less than twice the risk for malignant transformations.

In present study grade of elastosis was commonly associated with ductal epithelial hyperplasia,but was not stastically significant($p>0.05$). This was in accordance with study by Parfrey and Doyle et al[91] showed as periductal and interstitial elastosis in the Fibrocystic breast disease increased gradually with the severity of the epithelial hyperplasia.

In present study the grade of microvessel density correlates significantly with epithelial hyperplasia (p value< 0.05).A study by Guinebretiere J.M. et al and J E Bluff,SR Menakuru et al [101]showed similar results.

Breast cancer is the leading cause of cancer deaths among women. Results from experimental studies suggest that tumor progression and metastasis in breast cancer is angiogenesis dependant.

Angiogenesis has gained importance as a prognostic indicator in tumor progression. Tumor angiogenesis is generally measured by quantifying micro vessel density in sections immuno stained for vascular endothelial cell markers, such as CD34.

Takao Kato et al[104] found this marker to be a significant independent prognostic factor associated with long term survival in Japanese breast cancer patients, especially in node-negative patients.

In this present study majority of Invasive ductal carcinoma-nos type cases belong to age of 50 to 59 years.

A study conducted by ChungM et al [105] and Michel JKerin et al[106] showed similar results. A study by YildirimE,DalgicT et al[107] showed age was an independent prognostic factors in breast cancer.

In the present study predominant tumors were histologically grade II (46.60%) followed by grade III (33.40%) and grade I (20%) A study by Dalton LW, Page DL, Dupont WD et al[108] showed majority of tumors were histologically grade II followed grade I and grade III tumors.

In this present study there was no positive correlation between Microvessel density grade and age of Invasive ductal carcinoma cases. A study by Goulding et al, Costello et al also shows similar results.

A study by Curt Lundmark et al [88] showed grade 1 and grade 2 elastosis belong to age of 60 to 69 years and but no positive correlation between increasing age and grade of elastosis.

In this study high grade of Microvessel density was seen among grade III tumors followed by grade II and grade I tumors .In the present study a significant correlation (p value<0.05) is noted between histological grade and grade of Microvessel density. Similar to that found by Horak et al,[109] Weidner et al[71]and Bosari et al.

However, Goulding et al, Van Hoef et al[110] failed to find any association between Micro vessel density and prognosis.

In this present study grade 2 elastosis was seen among 50-59 years age group,grade 1 elastosis among >60 years and grade 0 elastosis among age of 20-39 years.A study by Curt Lundmark et al[88] showed that grade 1 and grade 2 elastosis was common between 60-69 years of age.

In this present study the correlation between histological grading and elastosis grade deos not apper to be a prognostic significance.But study by Jackson and Orr et al[90] found that grade II and grade III tumors showed 69.9% and 63.1% of elastosis respectively.

SUMMARY

The present study was conducted at Coimbatore medical college, Coimbatore during the year of April 2013 to july2014, titled as "**AN ASSESSMENT OF ANGIOGENESIS AND ELASTOSIS IN FIBROCYSTIC BREAST DISEASE AND INVASIVE BREAST CARCINOMA".** It is a descriptive study consists of thirty cases of fibrocystic breast diseases and thirty cases of invasive ductal carcinoma. Immunohistochemical study for CD34 was done for all the cases to grade the microvessel density. In addition Verhoeff-VanGieson stain was done to grade the elastosis. Patients who had underwent preoperative neo adjuvant chemotherapy and radiotherapy were excluded. Statistical analysis was done and compared with similar previous studies.

The present study showed the following results:

1. Majority of the cases of fibrocystic breast disease were in to the age group between 20-39 years.

2. Most common morphological changes of fibrocystic disease were cyst formation and usual ductal epithelial hyperplasia.

3. The association between ductal epithelial hyperplasia and grade of microvessel density was statistically significant.($p<0.05$)
4. The association between ductal epithelial hyperplasia and grade of elastosis was not statistically significant.(p-value>0.05)
5. Most common age of presentation of invasive duct breast carcinoma –Nos type were 50-59 years.
6. Most of the invasive duct breast carcinoma –Nos type were grade II tumors (46.6%).
7. The correlation between grade of microvessel density and age of invasive ductal carcinoma was not statistically significant.
8. The correlation between grade of elastosis and the age of Invasive duct carcinoma-Nos type cases was not statistically significant.Which may be , probably due to inadequate sampling.
9. The correlation between histological grade of invasive ductal carcinoma-Nos type and grade of microvessel density was statistically significant($p<0.05$) in contrast to elastosis which was statistically insignificant.

CONCLUSION

To conclude the available prognostic factors in breast carcinoma have been studied extensively.Angiogenesis in breast carcinoma has attracted interest as a prognostic indicator.The study of microvessel density using CD34 as one of the novel marker, has a promising role in targeted therapy.

Tumors lacking elastosis have showed lower rate of response to endocrine therapy. So, this might be useful as predictive marker of response to endocrine therapy.

BIBILIOGRAPHY

1. Wheater's functional histology , fifth edition page no: 387-388
2. Rosai and Ackerman's surgical pathology, Elsevier 10th edition vol2: pg-1673-1722.
3. Kim Y B,Hwang T S et . correlation of tumor grade and other prognostic factors in invasive breast carcinomas.journal of Korean cancer Association1994;26(6):926-35.
4. Pual peter Rosens Breast pathology .Third edition .pg -358-405
5. Haybittle J L,Blamey R W,Elston C W et al 1982 A Prognostic index in primary breast cancer.Br J Cancer 45:361-366.
6. NemotoT,Vana J et al.Management and survival of female breast cancer;results of a National survey by the American college of surgeons.cancer 1980;45:2917-24.
7. John .S.Meyer, Consulo Alvariz Breast carcinoma malingnancy grading by Bloom-Richardson system vs proliferation index: Reproducibility of grade and advantages of proliferation index; Modern pathology 2005; 18: 1069-1078.
8. Consensus statement: Treatment of early-stage breast cancer-National institute of Health consensus Development Panel. J Natl Cancer inst Monogr,1992;11:1-5.

9. Sternberg, Stacey E.Mills,Darryl Carter et al .Sternberg's Diagnostic surgical pathology. Fifth edition;vol 1:285-345.

10. Van Deist PJ, Baak JPA, Matze-cok P et al.Reproducibility of mitosis counting in 2469 breast cancer specimens; Result from multicentric Morphometric Mammary carcinoma project. Human Pathology 1992;23:603-607.

11. Frances P.O' Malley, Sarah .E. Pinder,Anna Marie Mulligan Breast Pathology: Foundations in Diagnostic pathology second edition;260-271.

12. Lester SC. The breast.In:Robbins and cotran, pathologic basis of disease.Kumar V, Abbas,A K ,Fausto N, editors.8th edition.page-

13. Rosee PP.The pathological classification of human mammary carcinoma: past,present,and future.Ann Clin Lab Sci 1979:9:144-56.

14) Sondik EJ: Breast cancer trends. Incidence, mortality, and survival. Cancer 1994; 74:995-999.

15) Armstrong K, Eisen A, Weber B: Assessing the risk of breast cancer. N Engl J Med 2000; 342:564-571.

16) Skolnick MH, Cannon-Albright LA: Genetic predisposition to breast cancer. Cancer 1992; 70:1747-1754.

17) Tulinius H, Sigvaldason H, Olafsdottir G: Left and right sided breast cancer. Pathol Res Pract 1990; 186:92-94.

18. Broët P, de la Rochefordière A, Scholl SM, Fourquet A, Massen V, Durand J-C, Pouillart P, Asselain B: Contralateral breast cancer. Annual incidence and risk parameters. J Clin Oncol 1995; 13:1578-1583.

19. Fletcher SW, Elmore JG: Mammographic screening for breast cancer. N Engl J Med 2003; 348:1672-1680.

20. Schwartz GF, Carter DL, Conant EF, Gannon FH, Finkel GC, Feig SA: Mammographically detected breast cancer. Nonpalpable is not a synonym for inconsequential. Cancer 1994; 73:1660-1665.

21. Arisio R, Cuccorese C, Accinelli G, Mano MP, Bordon R, Fessia L: Role of fine-needle aspiration biopsy in breast lesions: analysis of a series of 4,110 cases. Diagn Cytopathol 1998; 18:462-467.

22. Ballo MS, Sneige N: Can core needle biopsy replace fine-needle aspiration cytology in the diagnosis of palpable breast carcinoma? A comparative study of 124 women. Cancer 1996; 78:773-777.

23. Ferreiro JA, Gisvold JJ, Bostwick DG: Accuracy of frozen-section diagnosis of mammographically directed breast biopsies. Results of 1,490 consecutive cases. Am J Surg Pathol 1995; 19:1267-1271.

24. Collins LC, Achacoso N, Nekhlyudov L, Fletcher SW, Haque R, Quesenberry Jr CP, Puligandla B, Alshak NS, Goldstein LC, Gown AM, Schnitt SJ, Habel LA: Relationship between clinical and

pathologic features of ductal carcinoma in situ and patient age: an analysis of 657 patients. Am J Surg Pathol 2009; 33:1802-1808.

25. Moriya T, Silverberg SG: Intraductal carcinoma (ductal carcinoma in situ) of the breast. A comparison of pure noninvasive tumors with those including different proportions of infiltrating carcinoma. Cancer 1994; 74:2972-2978.
26. Bartow SA, Black WC, Waeckerlin RW, Mettler FA: Fibrocystic disease. A continuing enigma. Pathol Annu 1982; 17(Pt 2):93-111.
27. Consensus Meeting, Oct 3 to 5, 1985, New York, Cancer Committee of the College of American Pathologists: Is 'fibrocystic disease' of the breast precancerous?. Arch Pathol Lab Med 1986; 110:171-173.
28. Minkowitz S, Hedayati H, Hiller S, Gardner B: Fibrous mastopathy. A clinical histopathologic study. Cancer 1973; 32:913-916.
29. Ory H, Cole P, MacMahon B, Hoover R: Oral contraceptives and reduced risk of benign breast diseases. N Engl J Med 1976; 294:419-422.
30. Azzopardi JG: Problems in breast pathology. (consulting ed.) In: Bennington JL, ed. Major problems in pathology, vol. 11. Philadelphia: W.B. Saunders; 1979.
31. Washington C, Dalbegue F, Abreo F, Taubenberger JK, Lichy JH: Loss of heterozygosity in fibrocystic change of the breast: genetic

relationship between proliferative lesions and associated carcinomas. Am J Pathol 2000; 157:323-329.

32. Tse GM, Tan PH, Lau KM, de Andrade VP, Lui PC, Vong JS, Chaiwun B, Lam CC, Yu AM, Moriya T: Breast cancer in the elderly: a histological assessment. Histopathology 2009; 55:441-451.
33. Azzopardi JG, Laurini RN: Elastosis in breast cancer. Cancer 1974; 33:174-183.
34. Carstens PHB, Huvos AG, Foote Jr FW, Ashikari R: Tubular carcinoma of the breast. A clinicopathologic study of 35 cases. Am J Clin Pathol 1972; 58:231-238.
35. Venable JG, Schwartz AM, Silverberg SG: Infiltrating cribriform carcinoma of the breast. A distinctive clinicopathologic entity. Hum Pathol 1990; 21:333-338.
36. Chinyama CN, Davies JD: Mammary mucinous lesions: congeners, prevalence and important pathological associations. Histopathology 1996; 29:533-539.
37. Gaffey MJ, Mills SE, Frierson Jr HF, Zarbo RJ, Boyd JC, Simpson JF, Weiss LM: Medullary carcinoma of the breast. Interobserver variability in histopathologic diagnosis. Mod Pathol 1995; 8:31-38.

38. Nassar H, Qureshi H, Volkanadsay N, Visscher D: Clinicopathologic analysis of solid papillary carcinoma of the breast and associated invasive carcinomas. Am J Surg Pathol 2006; 30:501-507
39. Chen L, Fan Y, Lang RG, Guo XJ, Sun YL, Cui LF, Liu FF, Wei J, Zhang XM, Fu L: Breast carcinoma with micropapillary features: clinicopathologic study and long-term follow-up of 100 cases. Int J Surg Pathol 2008; 16:155-163.
40. Abati AD, Kimmel M, Rosen PP: Apocrine mammary carcinoma. A clinicopathologic study of 72 cases. Am J Clin Pathol 1990; 94:371-377.
41. Akhtar M, Robinson C, Ali MA, Godwin JT: Secretory carcinoma of the breast in adults. Light and electron microscopic study of three cases with review of the literature. Cancer 1983; 51:2245-2254
42. Righi L, Sapino A, Marchiò C, Papotti M, Bussolati G: Neuroendocrine differentiation in breast cancer: established facts and unresolved problems. Semin Diagn Pathol 2010; 27:69-76.
43. Yamaguchi R, Horii R, Maeda I, Suga S, Makita M, Iwase T, Oguchi M, Ito Y, Akiyama F: Clinicopathologic study of 53 metaplastic breast carcinomas: their elements and prognostic implications. Hum Pathol 2010; 41:679-685.
44. Weidner N, Semple JP: Pleomorphic variant of invasive lobular c arcinoma of the breast. Hum Pathol 1992; 23:1167-1171

45. Shimizu S, Kitamura H, Ito T, Nakamura T, Fujisawa J, Matsukawa H: Histiocytoid breast carcinoma: histological, immunohistochemical, ultrastructural, cytological and clinicopathologic studies. Pathol Int 1998; 48:549-556.
46. Dixon JM, Anderson TJ, Page DL, Lee D, Duffy SW: Infiltrating l obular carcinoma of the breast. Histopathology 1982; 6:149-161
47. Azzopardi JG: Problems in breast pathology. (consulting ed.) In: Bennington JL, ed. Major problems in pathology, vol. 11. Philadelphia: W.B. Saunders; 1979.
48. Prasad ML, Osborne MP, Giri DD, Hoda SA: Microinvasive carcinoma (T1mic) of the breast: clinicopathologic profile of 21 cases. Am J Surg Pathol 2000; 24:422-428.
49. Cao Y, Paner GP, Rajan PB: Sentinel node status and tumor characteristics: a study of 234 invasive breast carcinomas. Arch Pathol Lab Med 2005; 129:82-84.
50. Kinne DW: Staging and follow-up of breast cancer patients. Cancer 1991; 67:1196-1198.
51. Bonadonna G, Valagussa P, Brambilla C, Moliterni A, Zambetti M, Ferrari L: Adjuvant and neoadjuvant treatment of breast cancer with chemotherapy and/or endocrine therapy. *Semin Oncol* 1991; 18:515-524.

52. Mansfield CM, Krishnan L, Komarnicky LT, Ayyangar KM, Kramer CA: A review of the role of radiation therapy in the treatment of patients with breast cancer. *Semin Oncol* 1991; 18:525-535. 1237.
53. Walford N, ten Velden J: Histiocytoid breast carcinoma. An apocrine variant of lobular carcinoma. Histopathology 1989; 14:515-522
54. Eltorky M, Hall JC, Osborne PT, el Zeky F: Signet-ring cell variant of invasive lobular carcinoma of the breast. A clinicopathologic study of 11 cases. *Arch Pathol Lab Med* 1994; 118:245-248.
55. Fisher ER, Gregorio RM, Redmond C, Fisher B: Tubulolobular invasive breast cancer. A variant of lobular invasive cancer. *Hum Pathol* 1977; 8:679-683.
56. Barnes DM, Hanby AM: Oestrogen and progesterone receptors in breast cancer: past, present and future. *Histopathology* 2001; 38:271-274.
57. Cifuentes N, Pickren JW: Metastases from carcinoma of mammary gland. An autopsy study. *J Surg Oncol* 1979; 11:193-205.
58. Cao Y, Paner GP, Rajan PB: Sentinel node status and tumor characteristics: a study of 234 invasive breast carcinomas. *Arch Pathol Lab Med* 2005; 129:82-84.
59. Cserni G: Evaluation of sentinel lymph nodes in breast cancer. *Histopathology* 2005; 46:697-702.

60. Adami H-O, Malker B, Holmberg L, Persson I, Stone B: The relation between survival and age at diagnosis in breast cancer. *N Engl J Med* 1986; 315:559-563.
61. Biesterfield S, Noll I, Noll E, Wohltmann D, Blocking A: Mitotic frequency as a prognostic factor in breast cancer. *Hum Pathol* 1995; 26:47-52.
62. Bloom HJG, Richardson WW: Histological grading and prognosis in breast cancer. A study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer* 1957; 11:359-377.
63. Bloom HJG, Richardson WW, Harris ED: Natural history of untreated breast cancer. Comparison of untreated cases according to histological grade of malignancy. *Br Med J* 1962; 2:213-221.
64. Bogomoletz WV: Elastosis in breast cancer. *Pathol Annu* 1986; 21(Pt 2):345-366.
65. Bosari S, Lee AK, De Lellis RA, Wiley BD, Heatley GJ, Silverman ML: Microvessel quantitation and prognosis in invasive breast carcinoma. *Hum Pathol* 1992; 23:755-761.
66. Chung M, Chang HR, Bland KI, Wanebo HJ: Younger women with breast carcinoma have a poorer prognosis than older women. *Cancer* 1996; 77:97-103.
67. Dalton LW, Page DL, Dupont WD: Histologic grading of breast carcinoma. A reproducibility study. Cancer 1994; 73:2765-2770.

68. Fisher B, Slack NH, Bross IDJ: Cancer of the breast. Size of neoplasm and prognosis. *Cancer* 1969; 24:1071-1080.
69. Gallenberg MM, Loprinzi CL: Breast cancer and pregnancy. *Semin Oncol* 1989; 16:369-376.
70. Rennert G, Bisland-Naggan S, Barnett-Griness O, Bar-Joseph N, Zhang S, Rennert HS, Narod SA: Clinical outcomes of breast cancer in carriers of BRCA1 and BRCA2 mutations. *N Engl J Med* 2007; 357:115-123.
71. Weidner N: Intratumor microvessel density as a prognostic factor in cancer. *Am J Pathol* 1995; 147:9-19.
72. Weidner N, Semple JP, Welch WR, Folkman J: Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. *N Engl J Med* 1991; 324:1-8.
73. World Health Organization classification of tumours of breast and female gential organs page no: 9-110
74. Elston C, Ellis I. Pathological prognostic factors in breast carcinoma: The value of histological grade in breast cancer; Experience from a large study with long term followup. Histopathology 1991;19:403-10
75. Kim Y B,Hwang T S et . correlation of tumor grade and other prognostic factors in invasive breast carcinomas.journal of Korean cancer Association1994;26(6):926-35.

76. Haybittle J L,Blamey R W,Elston C W et al 1982 A Prognostic index in primary breast cancer.Br J Cancer 45:361-366.
77. Elston C W,Gresham G A,Rao G S etal. The Cancer Research Compaign Trial for early breast cancer- pathological aspects , Br J Cancer 1980 45:665-69.
78. Gold RH,Main G, Zippin C et al .Infiltrarion of mammary carcinoma as a indicators of axillary metastasis.A preliminary report .Cancer 1972:29;35-40.
79. Carter D ,Pipkin RD,Shepard RHet al .Relationship of necrosis and tumor border to lymph node metastasis and 10 year survival in carcinoma the breast.Am J surg 1978:2;39-46.
80. O Rourke S, Galea M H et al . An audit of local recurrence after s imple mastectomy.Br J Surg 1994,81;386-89.
81. Frances P.O' Malley, Sarah .E. Pinder,Anna Marie Mulligan Breast Pathology: Foundations in Diagnostic pathology second edition;260-271.
82. Jwan .F.Simpson Robert gray et al. Prognostic value of Histologic grade and proliferative activity in axillary node –positive breast cancer. Journal of clinical oncology 2000;18:2059-69.
83. Blamey R W,Davies C J,Elston C Wet al .Prognostic factors in breast cancer: the formation of prognostic index.Clin Oncol 5:22.

84. Fritz rank et al ,MD,Per, Dombernowsky et al .Histologic Malignancy of Grading of Invasive Ductal carcinoma .Cancer 1987;60:1299-1305.

85. Kupio T,Collan ,T.Still more about counting mitosis. Human Pathol 1996;27:1110-1111.

86. Folkman J (1971) Tumor angiogenesis : therapeutic implications. N Engl J Med 285:1182-1186.

87. Maha Mohammed Amin,Zeeinab et al.Indian Journal of pathology and microbiology.

88. Curt Lundmark .Breast Cancer and Elastosis.dep of pathology,danderyd hospital ,danderyd,Sweden.july3,1972:1195-1201.

89. Parfrey NA,Doyle CT.Elastosis in benign and malignant breast disease.Hum Pathol .1985 jul; 16(7):674-6.

90. Jackson,J .G ,and Orr,J.W.The ducts of carcinomatous breasts, with particular reference to connective –tissue changes .J. Pathol.Bacteriol.74:265-273,1957.

91. Parfrey NA,Doyle CT Elastosis in benign and malignant breast disease.Hum Pathol .1985 jul; 16(7):674-6.

92. Guinebretiere JM,Le Monique G,Gavoille A et al: Angiogenesis and risk of breast cancer in women with fibrocystic disease.JNatl Cancer inst 86:635-636,1994.

93. Holland R , Peterrse JL, Millis RR, Eusebi V , Faverly D, van de Vijver MJ, Zafrani B.Ductal carcinoma insitu . A proposal for a new classification.Semin Diagn Pathol 1994,11:167-180.

94. Carter D,Smith RRL,carcinoma in situ of the breast . Cancer 1977,40:1189-1193.

95. Ueng SH,Mezzetti T ,Tavassoli FA . Papillary neoplasms of the breast ; a review . Arch Pathol Lab Med 2009,133:893-907

96. Parfrey NA,Doyle CT.Elastosis in benign and malignant breast disease.Hum Pathol .1985 jul; 16(7):674-6.

97. J.R.W.M.Masters R.R. MILLIS. R.J.B.King and R.D.Rubens Elastosis and response to endocrine therapy in human breast cancer.

98. Ademer Longatto Filho ,Jose Manuel Lopes, and Fernando et al Angiogenesis and breast cancer.Jou of Onco 10.1155/ 2010/576384.

99. M.D.Safwat,F.Habib, A.Elayat et al .morphometric and immunohistochemical study of angiogenic marker expressions in invasive ductal carcinomas of the human breast.2009:vol68 ,no3 pp144-155

100. Jackson,J .G ,and Orr,J.W.The ducts of carcinomatous breasts, with particular reference to connective –tissue changes .J. Pathol.Bacteriol.74:265-273,1957.

101. Guinebretiere JM,Le Monique G,Gavoille A et al: Angiogenesis and risk of breast cancer in women with fibrocystic disease.JNatl Cancer inst 86:635-636,1994.

102. Shaaban AM, Sloane JP : Fibrocystic disease. A continuing enigma. Pathol Annu 1982;17(pt) :93-111.

103. YildirimE,DalgicT et 104. Takao Kato, Shingo Kameoka, Tsunehito Kimura, Naohiro Soga and Yutaka Abe. Angiogenesis as a predictor of long-term survival for 377 Japanese patients with breast cancer. Breast cancer Research and Treatment 2001;70:65-7

105. ChungM ,Prognosis and Risk factors of mammary carcinoma.Cancer journal patho.12:123-31.

106. Michel JKerin ,Chung M,Prognosis and Risk factors breast cancer in review.Cancer journal patho.12:123-31.

107. YildirimE,DalgicT Breast cancer risk and prognosis . pathol journal.15: 1267-69.

108. Doyle CT Elastosis in benign and malignant breast disease.Hum Pathol .1985 jul; 16(7):674-6.

109. Horak ER, Leek R, Klenk N, LeJeune S, Smith K, Stuart N, Greenall M, Stepniewska K, Harris AL. Angiogenesis, assessed by platelet/ endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. Lancet 1992;340:1120-4.

110. Goulding H, Abdul Rashid NF, Robertson JF, Bell JA, Elston CW, Blamey RW, Ellis IO. Assessment of angiogenesis in breast carcinoma: an important factor in prognosis. Hum Pathol 1995;26:1196-200.

ANNEXURE-I

PROFORMA

Name: Age:

Ward: IP/OP No:

Address:

Presenting complaints:

Breast lump

Pain

Nipple discharge

Skin ulceration

Duration of presenting complaint:

Past history:

History of previous surgeries for breast lump

History of chemotherapy/Radiotherapy

History of breast lump in other breast

Family history:

Personal history:

Diet

Menstrual history

Breast feeding history

General examination

Nourishment: Built: Consious:

Pallor: Jaundice: Cyanosis: Clubbing:

PR: RR: BP: Febrile/Afebrile:

Lymphadenopathy: Edema:

Local examination of the breast

Side: Right/Left

Quadrant:Upper/Outer/Inner/Lower/Central

Size of the tumor:

Fixity to skin: Yes/No Fixity to underlying Fascia: Yes/No

Examination of axillary lymph node:

Number of node:

Mobile/Fixed:

Size:

Group of nodes : Anterior/Posterior/lateral/apical

Gross examination of modified radical mastectomy specimen

Size of the specimen including skin, nipple & Areola:

Size of the tumor: <2cm/2-5cm/>5cm

Margins: Infiltrative/circumscribed

Quadrant: Outer/Inner/Central

Histologic diagnosis:

Any special type:

Lymph node status no: of positive nodes/no: of total nodes examined

Histologic grading

Tubule formation: 1/2/3

Nuclear pleomorphism: 1/2/3

Mitotis: 1/2/3

Histologic grade: I/II/III

MASTER CHART

S.NO	AGE	HPE NO	HISTOLOGIC DIAGNOSIS	HISTOLOGIC GRADE	MICROVESSEL DENSITY SCORE(GRADE)	ELASTOSIS GRADE
1	44	794/13	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE 0
2	38	989/13	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE 0
3	35	1247/13	FCD	NOT APPLICABLE	MILD GRADE	GRADE 1
4	40	1849/13	FCD	NOT APPLICABLE	SEVERE GRADE	GARDE2
5	21	1908/13	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE 1
6	28	2477/13	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE 0
7	32	2505/13	FCD	NOT APPLICABLE	MILD GRADE	GRADE1
8	25	2591/13	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE 0
9	36	2812/14	FCD	NOT APPLICABLE	MILD GRADE	GRADE 1
10	22	2813/14	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE 0
11	28	2905/14	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE2
12	31	3020/14	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE 0
13	42	3025/14	FCD	NOT APPLICABLE	MILD GRADE	GRADE 1
14	34	P51/14	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE 0
15	38	P79/14	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE1
16	42	P376/14	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE 2
17	38	P553/14	FCD	NOT APPLICABLE	MILD GRADE	GRADE0
18	45	P654/14	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE 1
19	43	P753/14	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE 0
20	26	P890/14	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE 1
21	42	P954/14	FCD	NOT APPLICABLE	MLID GRADE	GRADE1
22	56	P966/14	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE2
23	41	E394/14	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE0
24	49	E416/14	FCD	NOT APPLICABLE	MLID GRADE	GRADE1
25	58	E432/14	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE2
26	36	E502/14	FCD	NOT APPLICABLE	MILD GRADE	GRADE1
27	47	E550/14	FCD	NOT APPLICABLE	SEVERE GRADE	GRADE1
28	51	E573/14	FCD	NOT APPLICABLE	MODERATE GRADE	GRADE0
29	43	E632/14	FCD	NOT APPLICABLE	MILD GRADE	GRADE2

S.NO	AGE	HPE NO	HISTOLOGIC DIAGNOSIS	HISTOLOGIC GRADE	MICROVESSEL DENSITY SCORE(GRADE)	ELASTOSIS GRADE
30	46	E745/14	FCD	NOT APPLICABLE	SEVERE GRADE	GRDAE0
31	56	670/13	IDC-NOS	I	MODERATE GRADE	GRADE 0
32	55	767/13	IDC-NOS	II	SEVERE GRADE	GRADE 1
33	52	801/13	IDC-NOS	II	MODERATE GRADE	GRADE 0
34	65	858/13	IDC-NOS	III	SEVERE GRADE	GRADE 1
35	55	1029/13	IDC-NOS	II	MODERATE GRADE	GRADE 1
36	50	700/14	IDC-NOS	I	MODERATE GRADE	GRADE 0
37	40	793/14	IDC-NOS	II	SEVERE GRADE	GRADE 0
38	81	859/14	IDC-NOS	II	MODERATE GRADE	GRADE 2
39	50	860/14	IDC-NOS	III	SEVERE GRADE	GRADE 1
40	40	894/14	IDC-NOS	I	MILD GRADE	GRADE 0
41	52	931/14	IDC-NOS	III	SEVERE GRADE	GRADE 1
42	65	966/14	IDC-NOS	II	MILD GRADE	GRADE 0
43	45	1642/14	IDC-NOS	II	SEVERE GRADE	GRADE 0
44	38	1832/14	IDC-NOS	II	MODERATE GRADE	GRADE 0
45	66	1877/14	IDC-NOS	I	SEVERE GRADE	GRADE1
46	52	1895/14	IDC-NOS	II	MODERATE GRADE	GRADE 0
47	49	2039/14	IDC-NOS	II	SEVERE GRADE	GRADE 0
48	65	2179/14	IDC-NOS	III	MODERATE GRADE	GRADE 0
49	58	2050/14	IDC-NOS	II	MODERATE GRADE	GRADE 0
50	53	2135/14	IDC-NOS	II	SEVERE GRADE	GRADE 2
51	45	2179/14	IDC-NOS	II	SEVERE GRADE	GRADE 0
52	38	2240/14	IDC-NOS	III	MILD GRADE	GRADE 2
53	45	2296/14	IDC-NOS	I	SEVERE GRADE	GRADE 1
54	52	2461/14	IDC-NOS	III	SEVERE GRADE	GRADE 0
55	49	2462/14	IDC-NOS	II	MODERATE GRADE	GRADE 0
56	55	2516/14	IDC-NOS	III	SEVERE GRADE	GRADE 2
57	73	2622/14	IDC-NOS	II	MILD GRADE	GRADE 0
58	65	2676/14	IDC-NOS	III	SEVERE GRADE	GRADE 0
59	72	2762/14	IDC-NOS	I	MODERATE GRADE	GRADE 2
60	61	2773/14	IDC-NOS	III	MODERATE GRADE	GRADE 0

ABBREVIATIONS

FCD	-	Fibrocystic disease
IDC-NOS	-	Invasive ductal carcinoma-not otherwise specified
DCIS	-	Ductal carcinoma in situ
UDH	-	Usual ductal hyperplasia
ADH	-	Atypical ductal hyperplasia
WHO	-	World health organisation
AJCC	-	American joint committee classification
FNAC	-	Fine niddle aspiration cytology
MVD GRADE	-	Microvessel density grade
HG	-	Histological grade
EG	-	Elastosis grade
H&E STAIN	-	Haematoxyline and eosin stain
IHC	-	Immunohistochemistry
VVG STAIN	-	Verhoeff VonGieson stain
H&E SECTION	-	Haematoxyline and eosin section
VEGF	-	Vascular endothelial growth factor